Direct vasodilator effects of physiological hyperinsulin-aemia in human skeletal muscle

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Abstract. Systemic hyperinsulinaemia induces vasodilatation in human skeletal muscle. This effect is gradual in onset, and at low insulin levels not maximal until at least 3 h. To investigate whether the vasodilator response to insulin results from a direct vascular effect, we infused insulin directly into the cannulated brachial artery (perfused forearm technique) in a total of 30 experiments in 20 healthy, lean, normotensive volunteers. Local, intra-arterial, infusion of insulin (180 min, 0-3 mU dL⁻¹ forearm volume min⁻¹, n = 15, forearm venous insulin concentration approximately 540 pmol L⁻¹) induced a gradual increase in forearm blood flow (FBF; venous occlusion plethysmography) from 1-86 ± 0.17 to 3.64 ± 0.64 mL dL⁻¹ min⁻¹ after 180 min (ANOVA P < 0.001). Percentage increases in FBF after 60, 120 and 180 min averaged 14.4 ± 5.9, 59.4 ± 25.5 and 124.6 ± 51.2% respectively. Forearm glucose uptake increased from 0.24 ± 0.05 to a maximum of 1.98 ± 0.28 μmol dL⁻¹ min⁻¹ (P < 0.001). Furthermore, insulin infusion increased forearm lactate release and potassium uptake. In 10 out of these 15 individuals, the forearm glucose uptake was further increased in a second, separate, repeat experiment with concomitant intra-arterial infusion of glucose 5% (0.2 mL dL⁻¹ min⁻¹), resulting in forearm venous glucose concentrations of approximately 15 mmol L⁻¹. This combined infusion achieved a similar vasodilator response to the infusion of insulin alone. The individual vascular responses of the two paired experiments showed a strong correlation (r = 0.87, P < 0.01). In five subjects time and vehicle control experiments were performed, showing no changes in FBF or metabolism during the 180 min. We conclude that the slow vasodilator response to insulin (as observed during systemic infusion) can, at least partly, be explained by a direct vascular effect of insulin. Insulin-mediated skeletal muscle glucose uptake precedes this effect, but seems not to be an important determinant of the vasodilator response to insulin.

Keywords. Hyperglycaemia, insulin, vasodilatation.

Introduction

In acute experiments in humans, systemic insulin infusion with maintenance of euglycaemia, exerts a vasodilator effect in skeletal muscle [1-3]. This insulin-induced vasodilatation has been found to be reduced in disorders characterized by insulin resistance such as obesity [1], hypertension [14] and non-insulin-dependent diabetes mellitus (NIDDM) [5]. As insulin-mediated skeletal muscle vasodilatation significantly contributes to the disposal of glucose, a diminished vasodilator capacity in itself explains part of the decreased insulin sensitivity in insulin-resistant states [1,4,5]. Therefore, further research on the phenomenon of insulin-induced vasodilatation may be of clinical importance.

Controversy exists as to whether the vasodilator response to physiological hyperinsulinaemia results from a direct effect of insulin on the vascular wall. Whereas some authors have found an increase in blood flow in response to local insulin administration [6,7], others have not [8-10]. Based on the latter findings it has been concluded that the insulin-induced vasodilatation is centrally mediated [11,12]. In the present study we have carefully investigated the effect of regional hyperinsulinaemia on the putative vasodilatation in the skeletal muscle vascular bed, with special attention to the time course. Until now very little attention has been given to the time course of the insulin-induced vasodilator effect, which may be an important clue with respect to the controversial observations in the literature. From data of Laakso et al. [1], it is obvious that the vasodilator effect of systemic insulin infusion (physiological plasma insulin concentrations) is not maximal until 3 h of infusion, and that after 30 and 60 min, respectively, only approximately 10% and approximately 30% of the maximum effect has been reached. Recently, even slower increases in blood flow in response to insulin have been reported [13,14]. On the other hand, several studies in which insulin was administered locally have used only 20-30 min of infusion time [9,10,15].

Finally, as insulin-mediated glucose uptake may be an important determinant of the vasoactive effects of

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The experiments were performed with the subjects in years, non-smoking, absence of hypertension (office blood pressure <140/90 mmHg, measured after 5 min rest in the supine position), body mass index <25 kg m⁻². Participants used no medication, with the exception of oral contraceptives. The participants were selected by advertisement and received a payment. All subjects had a negative family history of diabetes and hypertension. All participants gave written informed consent. The experimental protocol was approved by the hospital ethics committee.

Methods

Subjects

The study group consisted of 20 healthy volunteers. All met the inclusion criteria: age between 18 and 35 years, non-smoking, absence of hypertension (office blood pressure <140/90 mmHg, measured after 5 min rest in the supine position), body mass index <25 kg m⁻². Participants used no medication, with the exception of oral contraceptives. The participants were selected by advertisement and received a payment. All subjects had a negative family history of diabetes and hypertension. All participants gave written informed consent. The experimental protocol was approved by the hospital ethics committee.

Procedures

The experiments were performed with the subjects in supine position after an overnight fast, in a quiet, temperature-controlled room (23-24°C). Under local anaesthesia (0.3-0.4 mL lidocaine HCl 20 mg mL⁻¹), a 20-gauge catheter (Angiocath, Becton Dickinson, Sandy, UT, USA) was inserted into the left 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 the electronically integrated area under the brachial arterial pulse-wave curve. The arterial line was kept patent with saline infusion (3 mL h⁻¹ with 2 U heparin mL⁻¹ added). In the same arm a catheter (Venflon, 20G, 32 mm) was inserted retrogradely into a deep forearm vein to obtain venous blood samples. On the contralateral side an identical catheter was inserted into a large forearm vein for blood sampling.

Forearm volume (FAV) was measured with the water displacement method. Forearm blood flow (FBF) was measured simultaneously in both arms, with the arms elevated just above heart level, using mercury-in-silastic strain-gauge venous occlusion plethysmography as previously described [17]. FBF was measured in mL dL⁻¹ FAV min⁻¹, and in the text abbreviated to mL dL⁻¹ min⁻¹. One minute before the start of the measurements, a wrist cuff was inflated to 100 mmHg above systolic blood pressure. The collecting cuff around the upper arm was inflated to a pressure of 40 mmHg during eight heart cycles using a Hokanson E20 rapid cuff inflator. The strain gauges were connected with the Hokanson EC4 plethysmographs. During a measurement period, 8-10 measurements of FBF were performed, each lasting approximately eight heart cycles.

Net uptake or release of glucose, lactate and potassium was calculated by simultaneous arterial and venous blood sampling (see calculations). Venous blood was sampled while the wrist cuffs remained inflated. Samples for insulin, potassium, lactate and pyruvate were taken at hourly intervals.

Protocols

Insulin (I) experiments. In 15 individuals, after complete instrumentation, at least 30 min of rest were included to obtain a steady state, after which baseline measurements were performed and repeated after 15 min. When FBF was stable, insulin (Actrapid, Novo-Nordisk, Denmark) was infused into the brachial artery, at a dose of 0.3 mU dL⁻¹ min⁻¹ (volume 50 µL dL⁻¹ min⁻¹) and continued for 180 min. Insulin 50 U mL⁻¹ was twice diluted in 100 mL of 0.9% NaCl each time with the addition of 2 mL of human albumin 20% (Central Laboratory of Blood transfusion, Amsterdam). FBF was measured after 5, 15 and thereafter every 15 min until 180 min.

Insulin + glucose (I + G) experiments. In 10 individuals, randomly selected out of the 15 who had participated in the first protocol, the entire experiment was repeated after at least 4 weeks, but now with the addition of 0.2 mL dL⁻¹ min⁻¹ glucose 5% infusion.

Control (C) experiments. In five different individuals, time and vehicle control experiments were performed, following exactly the same protocol, but now saline with the addition of albumin 20% (as mentioned above) was infused into the brachial artery for 180 min.

Analytical methods. Plasma glucose was measured in duplicate using the glucose oxidation method (Beckman glucose analyzer 2, Beckman Instruments, Fullerton, CA, USA).

Plasma insulin was measured with an in-house double antibody radioimmunoassay (interassay coefficient of variation 6.2%). For quantification of L-lactate (and pyruvate) in deproteinized blood (6% perchloric acid) an enzymatic assay (Lactate UV-kit; Boehringer Mannheim, Mannheim, Germany) with l-lactate dehydrogenase (L-LDH; EC 1.1.1.27) was performed. Because pyruvate concentrations remained at or below detection limit, these results were not used for further analyses. Potassium was measured with a standard procedure using a K⁺-ion selective electrode with a Hitachi 747 autoanalyzer (Boehringer Mannheim).

Calculations and data analysis. Forearm vascular resistance (FVR) was calculated by dividing MAP and FBF, and expressed in arbitrary units (AU). To correct for the increased fluid infusion in the second protocol, 0.2 was subtracted from the calculated FBF. For every 30-min period the mean of the previous 30-min measurements was calculated and used in the subsequent analyses. Forearm balances were
calculated as:

\[(\text{Concentration}_{\text{arterial}} - \text{concentration}_{\text{venous}}) \times \text{FPF}\]

Forearm plasma flow (FPF) = FBF \times (1 - \text{haematocrit})

Effects of insulin on haemodynamic parameters were analysed using one-way repeated measures ANOVA, with insulin as dependent factor. Post hoc \(t\)-tests were performed to test differences of the various time points from baseline. The effect of insulin alone was compared with the effect of insulin + glucose with the use of a two-way ANOVA for paired observations, with insulin and insulin + glucose as dependent variables. All other (mainly metabolic) data met requirements of normality and were statistically analysed with the use of Student’s \(t\)-test. Correlations were calculated using Spearman’s rank correlation tests. For the relation between venous insulin concentrations and forearm glucose extraction \((\text{GA}_1 - \text{GV}_1)/\text{GA}_1\), linear regression was performed and the coefficients of regression were used for further analysis. All statistical analyses were performed using the SPSS personal computer software package.

Results in tables and figures are expressed as mean ± SEM, unless otherwise indicated. Statistical significance was set at a value less than 0.05 (two-sided). NS means not significant.

Results

Baseline characteristics

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

Vascular responses to local hyperinsulinaemia

Insulin infusion led to deep venous forearm insulin concentrations of 549 ± 43, 497 ± 53 and 404 ± 50 pmol L\(^{-1}\) after 60, 120 and 180 min respectively, whereas arterial levels did not increase: 57 ± 5, 57 ± 4, 54 ± 4 and 49 ± 4 pmol L\(^{-1}\) (baseline, 60, 120 and 180 min respectively).

Local infusion of insulin induced a highly significant increase in forearm blood flow over time, from 1.86 ± 0.17 to 3.64 ± 0.64 mL dL\(^{-1}\) min\(^{-1}\) (ANOVA \(P < 0.001\)). This vasodilatation did not occur before 15 min, and was significantly different from baseline after 30 min. As can be seen in Fig. 1, the increase in FBF was gradual and even after 180 min, FBF did not seem to have reached a steady state. The increase in flow was attended by a significant decrease in FVR throughout levels at 30 min and increased slightly afterwards (see Fig. 1). Calculated forearm glucose uptake increased from 0.24 ± 0.05 to a maximum of 1.98 ± 0.28 \(\mu\)mol dL\(^{-1}\) min\(^{-1}\) (at 30 min, \(P < 0.001\)) and decreased slightly afterwards to 1.29 ± 0.19 after 180 min (\(P < 0.01\) vs. 30 min).

Metabolic responses to local hyperinsulinaemia

Arterial forearm glucose values did not change, whereas venous forearm glucose levels decreased to trough levels at 30 min and increased slightly afterwards (see Fig. 1). Calculated forearm glucose uptake increased from 0.24 ± 0.05 to a maximum of 1.98 ± 0.28 \(\mu\)mol dL\(^{-1}\) min\(^{-1}\) (at 30 min, \(P < 0.001\)) and decreased slightly afterwards to 1.29 ± 0.19 after 180 min (\(P < 0.01\) vs. 30 min).

Insulin infusion increased forearm lactate release (from baseline 70 ± 27 to 340 ± 52 nmol dL\(^{-1}\) min\(^{-1}\) after 120 min, \(P < 0.001\)) and uptake of potassium (from -0.17 ± 0.05 to 0.24 ± 0.15 \(\mu\)mol dL\(^{-1}\) min\(^{-1}\), \(P < 0.05\), see also Table 2). No significant correlations were found between changes in metabolic parameters and blood flow changes. Inherent to its metabolic effect, a positive relationship was observed between deep venous insulin concentrations and glucose extraction across the forearm vascular bed (\(P < 0.01\)).

Effects of the addition of glucose

Infusion of glucose into the brachial artery led to venous glucose concentrations of approximately 15 mmol L\(^{-1}\) (13.8 ± 1.0, 14.2 ± 1.0, 14.4 ± 0.9, 15.9 ± 1.6, 15.2 ± 0.8 and 15.6 ± 1.8 mmol L\(^{-1}\), after 30, 60, 90, 120, 150 and 180 min respectively). Systemic venous glucose levels (measured at contralateral side) remained approximately 5 mmol L\(^{-1}\) (4.7 ± 0.1, 1996 Blackwell Science Ltd, *European Journal of Clinical Investigation*, 26, 772–778

<table>
<thead>
<tr>
<th>Table 1. Characteristics of participants (mean ± SD)</th>
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<tbody>
<tr>
<td><strong>Insulin alone</strong></td>
</tr>
<tr>
<td>Number (M/F)</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>BMI (kg m(^{-2}))</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
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<tr>
<td>Diastolic BP (mmHg)</td>
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<td>Fasting glucose (nmol L(^{-1}))</td>
</tr>
<tr>
<td>Arterial</td>
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<td>Venous</td>
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LOCAL INSULIN INDUCES VASODILATATION

4.8 ± 0.1 and 4.9 ± 0.1 mmol L⁻¹, after 60, 120 and 180 min respectively). Venous insulin levels were comparable with those in the first set of experiments (568 ± 67, 637 ± 67 and 566 ± 89 pmol L⁻¹, after 60, 120 and 180 min respectively). The combination of hyperinsulinaemia and hyperglycaemia induced a significant increase in flow (FBFexperimental arm from 2.03 ± 0.29 to 2.79 ± 0.28 mL dL⁻¹ min⁻¹ after 180 min, ANOVA P < 0.01, see Fig. 2), whereas FBF in the control arm did not change (from 2.13 ± 0.47 to 2.34 ± 0.54 mL dL⁻¹ min⁻¹, P = NS).

Comparing the paired data sets of insulin alone vs. insulin + glucose infusion (n = 10), no significant difference in the FBF changes between the two experiments was observed (FBFexperimental arm, insulin alone (I): from 1.63 ± 0.12 to 1.78 ± 0.12, 2.04 ± 0.23, 2.66 ± 0.32 mL dL⁻¹ min⁻¹; insulin + glucose (I + G): from 2.03 ± 0.29 to 2.30 ± 0.24, 2.53 ± 0.23, 2.79 ± 0.28 mL dL⁻¹ min⁻¹, values after 60, 120 and 180 min respectively). Also the percentage increases in FBF of both paired experiments were not significantly different between the two experiments (increase after 30 min: I, 7.7 ± 5.2% vs. I + G, 21.5 ± 8.1% P = 0.12; after 90 min: I, 24.9 ± 12.0% vs. I + G, 24.9 ± 15.5% P = 1.00; after 150 min: I, 40.8 ± 17.2% vs. I + G, 40.3 ± 14.9% P = 0.97).

During I + G, forearm lactate release (P = 0.012) and potassium uptake (P = 0.086) reached higher levels than during I alone (see Table 2), indicating a local increase of metabolic pathways.

A significant correlation between the percentage increase in FBF during I alone and during concomitant I + G was established on various time points. In Fig. 3 the correlation at 90 min is shown: r = 0.87, P = 0.001.

**Table 2. Lactate release and potassium uptake at different time points (mean ± SE)**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Lactate release (nmol dL⁻¹ min⁻¹)</th>
<th>Potassium uptake (/mol dL⁻¹ min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>I + G</td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>0</td>
<td>70 ± 27</td>
<td>102 ± 47</td>
</tr>
<tr>
<td>60</td>
<td>257 ± 65</td>
<td>793 ± 194</td>
</tr>
<tr>
<td>120</td>
<td>340 ± 52</td>
<td>798 ± 165</td>
</tr>
<tr>
<td>180*</td>
<td>0.17 ± 0.05</td>
<td>0.14 ± 0.12</td>
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<tr>
<td></td>
<td>0.10 ± 0.10</td>
<td>0.28 ± 0.12</td>
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<td></td>
<td>0.32 ± 0.16</td>
<td>0.52 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>0.24 ± 0.15</td>
<td>0.48 ± 0.15</td>
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I, local hyperinsulinaemia alone; I + G, combination of local hyperinsulinaemia and hyperglycaemia; C, sham experiment.

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Discussion

The major new finding of the present study is that local insulin administration in the vascular bed of the human forearm skeletal muscle, leading to forearm deep venous insulin levels in the physiological range, induces a moderate, but reproducible, degree of vasodilatation. This, however, is slow in onset and preceded by maximal forearm glucose-uptake. In fact, the time course of the vasodilatation is very similar to that found with systemic insulin administration, leading to similar insulin levels [13]. Furthermore, artificial raising of glucose uptake by local hyperglycaemia did not influence the vasodilator response to insulin.

In recent years, various investigators, including ourselves, have shown the vasodilator capacities of insulin [1-3]. Interestingly, in various related disorders such as obesity, hypertension and NIDDM, characterized by insulin resistance [19,20], a decreased insulin-induced vasodilatation has also been reported [1,4,5]. The mechanism by which insulin exerts vasodilator effects could therefore be of great importance. Although not fully understood up to now, recent data seem to point towards a nitric oxide-dependent mechanism of action [7,21]. One of the controversies is whether the vasodilator response results from a direct effect on the vascular wall or from an indirect, possibly central, mechanism [22]. In favour of the latter hypothesis is the fact that several investigators were not able to show any vasodilatation when insulin was infused locally [8,10,23].

Until now little attention has been given to the time course of insulin-induced vasodilatation or to the relation between the speed of onset of the vasodilator effect and the insulin (and glucose) concentration. From the data available, it is clear that during euglycaemic systemic hyperinsulinaemia, insulin-induced vasodilatation is slow in onset and takes at least 3 h to obtain its maximal effect at physiological concentrations [1,13]. This is considerably longer than the time needed to reach maximal glucose uptake [1,16] and means that in studying blood flow responses to insulin, not only concentration but also duration of infusion may be critical (which will also hold true for local infusion). In keeping with this, Lundgren et al. [24], reported an increase in calf blood flow from 2.3 to 3.2 after 2 h, but even further to 4.4 mL dL⁻¹ min⁻¹ after 6 h of a fixed-dose insulin infusion.

Therefore it is obvious that in studying local vasodilator effects of insulin, sufficient time should be taken to allow insulin to exhibit its full effects, as we did in this study. This fact could explain why some authors were not able to identify any local action of insulin, as a number of investigators infused insulin for only 20-30 min [9,10,15]. Gelfand & Barrett [25] established a small increase in blood flow after 80 min, as did Neahrng et al. [26], and Jern [27] using a paired study approach (2-h local insulin infusion). It can be speculated that at higher insulin concentrations the vasodilator effect will appear earlier, and hence be found after 15-30 min [6,7].

In our study, the individual response to insulin, although reproducible, showed a high variability, ranging from no effect to more than doubling of the blood flow (Fig. 3). This high interindividual variation has also been reported by others and related to the relative forearm muscle content [14]. Thus, studies with small sample sizes, have a considerable risk of a type 2 statistical error, which could have further contributed to the controversial findings up to now.

Whereas we show in this study that at least part of insulin's vasodilator action can be explained by a local effect on the vascular wall, our study was not intended to explain the exact mechanism of insulin's effect. It seems that insulin-mediated glucose uptake precedes vasodilatation in skeletal muscle, but this does not mean that these two actions are coupled. In our own study, the addition of extra glucose, which must certainly have accelerated glucose uptake [28], had no additional effect on the change in vascular resistance. Only Edelman et al. have reported that blood flow increased with higher glucose levels during a constant insulin concentration, [16]. Their results were, however, derived from complex, sequential ("stepped") clamps, with a duration of approximately 8 h. Theoretically it may be possible that the changes
they observed were the consequence of prolonged exposure to insulin alone [24], and not of the higher glycemic level. Others have found no differences in flow in relation to different glycemic levels [28]. Furthermore, Vollenweider et al. [29] have shown that insulin (with glucose) infusion induced vasodilation, but fructose infusion alone, which increased carbohydrate oxidation comparably but had only minor effects on insulinaemia, did not stimulate skeletal muscle blood flow. As such it may be concluded that there is no firm evidence that hyperglycaemia augments insulin-induced vasodilatation.

In our study glucose uptake increased rapidly, but then slightly decreased during the subsequent experiment. During systemic hyperinsulinaemia, this decrease in glucose uptake over time is not observed. This finding is probably related to our experimental approach; owing to the increase in blood flow and the fixed insulin infusion rate, forearm insulin concentrations decreased, leading to a decreased glucose extraction. In favour of this explanation is the fact that we found a significant, positive, intra-individual correlation between venous insulin concentrations and glucose uptake. Therefore this secondary decrease in forearm glucose uptake will be related to the experimental model, and hence although partly inevitable, unphysiological.

During regional hyperinsulinaemia, venous plasma glucose concentrations decreased to hypoglycaemic levels. It is important to realize that this would also occur in euglycaemic clamps. The term euglycaemia refers to the arterial(ized) glucose concentration, but, owing to the stimulatory effect of insulin on glucose uptake, venous glucose concentrations will be considerably lower. Further, it has to be stressed that, because of the local approach, no systemic hypoglycaemia occurred in our studies. We have found, as have others [30], that during local insulin infusion lactate release increases. However, there was no correlation established between lactate release and vasodilatation. Furthermore, during the combination of hyperglycaemia and hyperinsulinaemia, lactate release further increased, suggesting more active metabolic pathways, but the increase in blood flow was not affected. Also others have argued that it is unlikely that lactate increase accounts for insulin's vasodilator effect [1].

In conclusion, we report that local hyperinsulinaemia leading to physiological insulin levels in the human forearm induces a slow-onset local vasodilatation that is preceded by maximal tissue glucose uptake. The time course of the effect seems to be similar to that found in studies in which insulin is infused systemically and indicates that the insulin-induced vasodilator effect is mediated at least partly at the level of the skeletal muscle. Although insulin-mediated skeletal muscle glucose uptake preceded the vasodilatory effect, it seems not to be an important determinant of the vasodilator response.

Acknowledgment
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