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Local Vasodilatation With Metacholine, But not With Nitroprusside, Increases Forearm Glucose Uptake

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Summary

Insulin is known to increase blood flow in parallel to glucose uptake in skeletal muscle. However, it is not known if an increase in blood flow by itself is associated with an increase in glucose uptake in the absence of hyperinsulinemia. To investigate further this matter, the effect of increased blood flow on forearm glucose uptake was studied in the fasting state during intra-arterial infusions of two different vasodilators, metacholine and nitroprusside, in 19 hypertensive subjects. Both metacholine (4 μg/min) and nitroprusside (10 μg/min) increased resting forearm blood flow, measured by venous occlusion plethysmography, to a similar degree (180 % and 170 %, respectively, $p<0.0001$ for both). However, metacholine infusion increased the forearm glucose uptake from 2.0±0.9 (S.D.) during rest to 5.5±3.0 umol/min/100 ml tissue ($p<0.0001$), while no significant change in glucose uptake was seen during nitroprusside infusion (2.3±1.4 μmol/min/100 ml tissue). In conclusion, vasodilatation induced by metacholine, but not by nitroprusside, increased glucose uptake in the forearm of hypertensive patients. Thus, an increase in forearm blood flow does not necessarily improve glucose uptake in the forearm during the fasting state.

Key words
Insulin • Blood flow • Glucose

Introduction

Insulin is known to increase blood flow in parallel to glucose uptake in skeletal muscles, the major site of insulin-mediated glucose disposal in the body (Laakso *et al.* 1990, Baron *et al.* 1993). However, it is unclear if an increase in blood flow in itself is associated with enhanced glucose uptake when blood flow is stimulated by other means than by insulin (Baron *et al.* 1994, Nuutila *et al.* 1996).

To investigate further this matter, the effect of an increased blood flow on forearm glucose uptake was studied in the fasting state during infusions of two different vasodilators namely metacholine, a potent stimulator of the release of nitric oxide (NO) from the endothelium, and nitroprusside, a NO-donator acting independently of the endothelium. Furthermore, as glucose uptake is also associated with transmembrane ion fluxes, the impact of ion balance during vasodilatation was studied with respect to glucose uptake.

Material and Methods

The studied sample consisted of 19 hypertensive men (aged 36-68 years, mean age 54, mean body mass index 27.3±3.4 kg/m^2 on regular medication with

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antihypertensive drugs (for basic characteristics and the use of different antihypertensive drugs, heredity of hypertension and smoking see Tables 1 and 2). With the exception of essential hypertension the patients were quite healthy. The primary aim of this investigation was to evaluate the endothelial function in a group of hypertensive patients on regular medication (Millgard *et al.* 1998). Antihypertensive medication was therefore not withdrawn except for the day of examination.

Table 1. Basic characteristics and metabolic variables at rest.

Age (years)	54±8
SBP (mm Hg)	143±19
DBP (mm Hg)	90±7
SaO_2 at rest (%)	95±2
SvO_2 at rest (%)	79±11
Arterial glucose (mmol/l)	5.1±15
Venous glucose (mmol/l)	4.7±1.5
Serum sodium (mmol/l)	139±1.7
Serum potassium (mmol/l)	4.1±0.25
Serum Ca^{2+} (mmol/l)	1.12±0.42
Serum Mg^{2+} (mmol/l)	0.53±0.054

Data are means ± S.D. SBP = systolic blood pressure DBP = diastolic blood pressure, SaO_2 = oxygen saturation in arterial blood, SvO_2 = oxygen saturation in venous blood

Table 2. Prevalence of different antihypertensive drugs, heredity of hypertension and smoking in the sample.

	Prevalence (%)
Beta-blockers	53
Calcium channel-blockers	68
Diuretics	21
ACE-inhibitors	63
Alpha-blockers	5
Heredity of hypertension	68
Smoking	26

The studies were performed in the fasting state on the subjects laying in the supine position in an air conditioned room maintained at a constant temperature (20 °C). An catheter was inserted into the brachial artery for regional infusion of metacholine or nitroprusside. A catheter was another placed in the deep antecubital vein. Basal forearm blood flow, arterial and venous oxygen saturation and concentrations of glucose, potassium, ionized calcium and ionized magnesium were recorded before and during two separate infusions. Both infusions were given in the course of 10 min with a 30 min wash-out period. The infused dosages were 4 μg/min for metacholine and 10 μg/min for nitroprusside. The drugs were given in random order and at a rate of 1 ml/min. The given dosages were known to induce similar increments in blood flow in healthy volunteers.

Forearm blood flow was measured by venous occlusion plethysmography. A mercury in-silastic strain gauge was placed on the upper third of the forearm, which rested comfortably slightly above the level of the heart. The strain gauge was coupled to a calibrated plethysmograph. Venous occlusion was achieved by a blood pressure cuff applied proximal to the elbow and inflated to 40 mm Hg by a rapid cuff inflator.

The experiments were performed in one arm while the contralateral arm served as control. Forearm blood flow was determined from the mean of at least five consecutive recordings. Glucose was measured by a Beckman glucose analyzer, oxygen saturation in arterial and venous blood was measured in a blood gas analyzer (Radiometer, Copenhagen, Denmark) and ions by an ion-sensitive electrode (Microlyte, Kone Instr., Finland). Glucose uptake was calculated from the product of forearm blood flow and the arterio-venous difference for glucose, while forearm oxygen uptake was calculated from the formula: blood flow * a-v difference of oxygen saturation * hemoglobin concentration * 1.38.

The study protocol was approved by the Ethics Committee of the University of Uppsala and informed consent was obtained from all participants.

The differences between the groups were calculated by factorial ANOVA. $p<0.05$ was regarded as significant.

Results

Forearm blood flow increased from resting values of 5.7±2.5 (S.D.) to 16.0±6.7 ml/min/100 ml tissue ($p<0.0001$) during infusion of nitroprusside and to 15.4±5.3 ml/min/100 ml tissue ($p<0.0001$) during infusion of metacholine (Fig. 1). The arterio-venous oxygen saturation difference decreased from a mean basal value of 16.1±10.7 to 4.8±2.8 % during infusion of metacholine ($p<0.0001$) and to 4.9±3.7 % during nitroprusside infusion ($p<0.0001$)

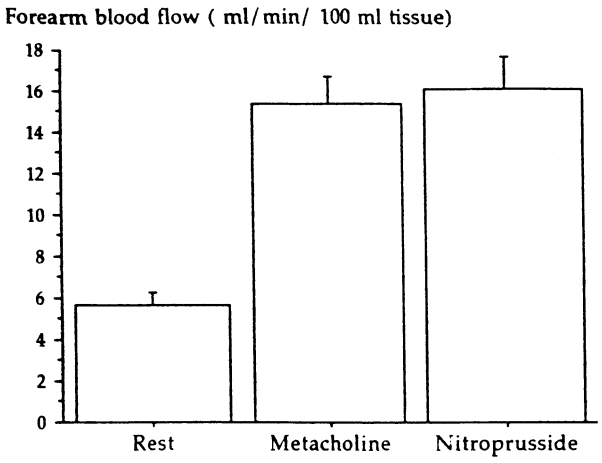

Fig. 1. *Forearm blood flow during rest and during local vasodilatation with metacholine and nitroprusside. Means ± S.E.M. are given. The differences in blood flow between the resting state and blood flow during metacholine and nitroprusside infusion are significant for p<0.001.*

While no significant change in the *arterio-venous glucose difference* was seen between the mean resting value to that obtained during metacholine infusion (0.37±0.19 mmol/l), nitroprusside induced a decrease in the mean arterio-venous difference for glucose from 0.39±0.17 during rest to 0.17±0.16 mmol/l (p<0.001).

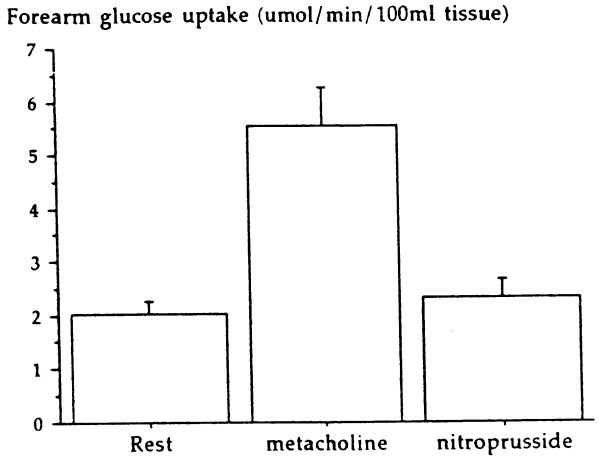

Fig. 2. *Forearm glucose uptake during rest and during local vasodilatation with metacholine and nitroprusside. The difference in glucose uptake between the resting state and during metacholine infusion is significant for p<0.001.*

The *forearm oxygen uptake* at rest (1.5±1.0 ml/min/100 ml tissue) remained on a similar level during infusion of metacholine and nitroprusside (1.3±0.9 and 1.5±1.4 ml/min/100 ml tissue, respectively).

However, the metacholine infusion increased *forearm glucose uptake* from 2.0±0.9 during rest to 5.5±3.0 μmol/min/100 ml tissue (p<0.0001), while no significant change in glucose uptake was seen during nitroprusside infusion (2.3±1.4 μmol/min/100 ml tissue) (Fig. 2). This change in forearm glucose uptake during metacholine infusion was not related to the type of antihypertensive medication, as evaluated by ANOVA or multiple regression analysis. No significant differences were found between smokers and non-smokers (5.3±3.2 vs 6.2±6.2 μmol/min/100 ml tissue, p = 0.68)

The glucose uptake during metacholine infusion was significantly correlated to the blood flow at rest (r = 0.62, p<0.01) (Fig. 3), while an inverse correlation was found between glucose uptake during metacholine infusion and body weight (r = –0.55, p<0.02).

The arterial and venous *levels of ions* did not change positively during infusions of the two drugs, but a significant inverse relationship was found between the venous potassium level and glucose uptake during metacholine infusion (r = –0.50, p<0.04).

No significant correlations were found between glucose uptake during infusion of metacholine and basal blood glucose levels, blood pressure, age or type of antihypertensive medication.

Discussion

The present study has shown that locally infused metacholine increased forearm glucose uptake, while nitroprusside did not, despite the fact that both drugs induced a similar increase in blood flow.

It has been reported that vasodilatation with metacholine given into the femoral artery increased glucose uptake during concomitantly induced hyperinsulinemia (Baron *et al.* 1994). On the contrary, other investigators have shown that vasodilatation with bradykinin did not alter limb glucose uptake (Nuutila *et al.* 1996). Thus, the present investigation has shown a similar picture that forearm glucose uptake is increased by one drug, but not by the other.

The increase in glucose uptake induced by metacholine in a previous study (Baron *et al.* 1994) was of the same magnitude as found in the present investigation. This was seen in spite of the fact that

hyperinsulinemia was present in the previous study using metacholine as vasodilator. In the present report, the insulin levels were likely to be low as vasodilatation was induced in the fasting state.

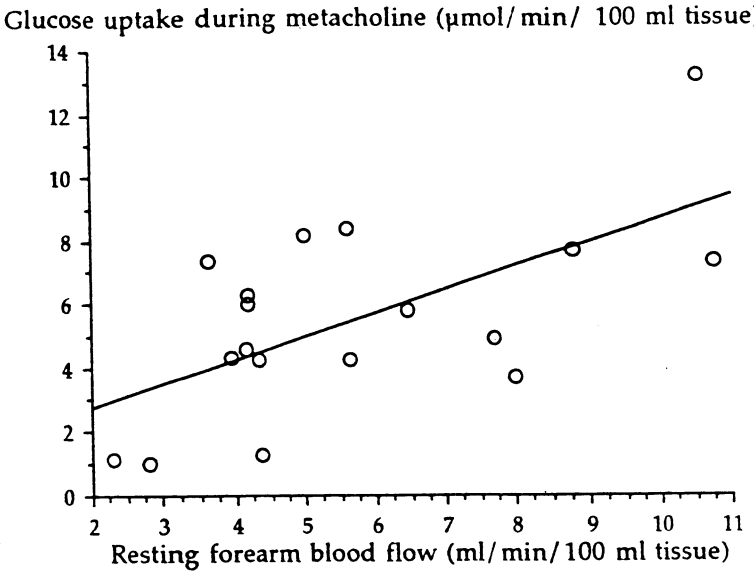

Fig. 3. *Relationship between forearm glucose uptake during vasodilatation with metacholine and the resting forearm blood flow ($r=0.62$, $p<0.01$).*

There are several possible explanations for the finding that metacholine increased glucose uptake. First, metacholine is an acetylcholine equivalent stimulating muscarinic receptors. However, there are no known reports indicating that muscarine receptor stimulation has any direct effects on glucose uptake (Baron *et al.* 1994). There is also a possibility that metacholine and nitroprusside dilate different types of vessels, although the net increase in blood flow measured by the venous occlusion plethysmographic technique is similar for both drugs. Metacholine might dilate vessels in such a way that the blood flow is directed towards a metabolically active tissue resulting in an uptake of glucose in the skeletal muscle, while nitroprusside might dilate other types of vessels without enhancing effective nutrient blood flow. This view is supported by some preliminary findings during measurements of tissue blood flow using microdialysis catheters by the ethanol clearance technique (Hickner *et al.* 1992) in which the increase of blood flow in skeletal muscle was substantially greater during infusion of metacholine than during infusion with nitroprusside (unpublished data). A third putative mechanism behind the finding of the increased uptake of glucose by metacholine could be due to activation of nicotine receptors inducing contraction in the skeletal muscle resulting in increased glucose uptake. However, this explanation seems unlikely since no parallel increase in oxygen consumption was seen and would be suspected if an increase in oxidative metabolism were induced by muscle contraction.

The fact that oxygen consumption was not increased during infusion of metacholine despite the marked increase in glucose uptake suggests that the increased glucose taken up in the skeletal muscle is not oxidized, but stored as glycogen. This is similar to what is seen when blood flow is increased by stimulation with insulin under resting conditions (Thiebaud *et al.* 1982).

The present study involved only hypertensive patients. This group of patients is known to have reduced glucose uptake during hyperinsulinemic conditions, as evaluated by the euglycemic hyperinsulinemic clamp (Ferrannini *et al.* 1987, Pollare *et al.* 1990). It has furthermore been shown that the vasodilating effects of insulin are impaired in subjects with a high blood pressure (Baron *et al.* 1993). However, in the previously cited study (Baron *et al.* 1994), showing stimulation of glucose uptake by metacholine infusion, the population studied was healthy and normotensive suggesting that this finding is not restricted to hypertensive subjects. In a recently published paper, vasodilation with nitroprusside did not increase glucose uptake (Natali *et al.* 1998).

The forearm glucose uptake during vasodilatation with metacholine was related to the blood flow at rest implying that subjects with a high resting blood flow have an ability to increase glucose uptake from the circulation when blood flow is increased by

certain procedures. Nevertheless, the glucose uptake during metacholine infusion was reduced in obese subjects. The glucose uptake was more than doubled when normal weight subjects (80 kg) were compared to those with a body weight of more than 100 kg. This is in accordance with previous data that insulin-mediated glucose uptake is diminished in obese subjects (Natali *et al.* 1998, Reaven 1988). This has recently been attributed to a defect in the ability of insulin to stimulate blood flow (Laakso *et al.* 1990), but the present study showed that the ability of skeletal muscle to remove glucose from the circulation is impaired in obese subjects, even when blood flow is increased by other means than by insulin.

The glucose uptake during metacholine infusion was inversely related to the circulating levels of potassium. It is well known that the circulating levels of potassium decrease during insulin-mediated glucose uptake due to stimulation of the Na^+-K^+-ATPase (De Fronzo *et al.* 1976). The present findings support the view that metacholine-induced glucose uptake is also associated with the decreased potassium levels.

In conclusion, vasodilatation induced by metacholine, but not by nitroprusside, increased glucose uptake in the human forearm. Thus, an increase in forearm blood flow does not necessarily improve glucose uptake in the forearm in the fasting state.

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