Induction of rat muscle insulin resistance by epinephrine is accompanied by increased interstitial glucose and lactate concentrations

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Summary Muscle glucose uptake and lactate release during β -adrenergic stimulation by epinephrine (epi) and β -adrenergic blockade by propranolol (prop) were investigated during an euglycaemic hyperinsulinaemic ($30 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) with or without added somatostatin ($0.1 \text{ } \mu\text{g}/\text{min}$; pancreatic) clamp in female rats. To assess the interstitial insulin, glucose and lactate concentrations, microdialysis was done in the medial femoral muscle in both legs. The influence of muscle skeletal blood flow on interstitial insulin, glucose and lactate was examined with the microsphere technique, using ^{57}Co -microspheres. Epinephrine decreased glucose infusion rate by about 75 % ($p < 0.0001$) and increased concentrations of interstitial glucose by about 35 % ($p < 0.001$) and lactate by about 65 % ($p < 0.01$). Plasma insulin concentration increased during β -adrenergic stimulation by about 25 % ($p < 0.05$) whereas the interstitial insulin concentration was unchanged. Muscle blood flow in the

hindlimb was considerably enhanced by about 130 %, ($p < 0.001$) by epinephrine. Infusion of propranolol totally abolished all the above effects induced by epinephrine. The data show that insulin resistance and vasodilation induced by β -adrenergic stimulation with epinephrine is accompanied by increased interstitial glucose as well as lactate concentrations in muscle. The increased interstitial glucose concentration is the result of a decreased cellular uptake of glucose together with an increased capillary delivery of glucose by vasodilation. It is concluded that the severe cellular resistance to insulin induced by epinephrine could not be overcome either by the increased insulin secretion or by vasodilation. [Diabetologia (1998) 41: 1467–1473]

Keywords Insulin resistance, epinephrine, microdialysis, interstitial fluid, blood flow, rat.

Epinephrine and β -adrenergic stimulation rapidly inhibit insulin-mediated glucose uptake by induction of insulin resistance mainly in the skeletal muscle [1, 2]. The insulin signalling pathway may be altered by β -adrenergic stimulation both by cAMP and cAMP independent mechanisms [3]. An inhibitory action at multiple sites is suggested since the insulin signal is disturbed at the receptor level [4] as well as in the tyrosine kinase [5], the glucose transporter protein [6] and the glycolytic pathway [7]. Moreover, induction of lipolysis by β -adrenergic stimuli leads to decreased

oxidation of glucose through the action of the Randle cycle [8].

The glucose transporter protein is considered to be rate-limiting for insulin-stimulated glucose uptake in the muscle. Moreover, recent research has emphasized the importance of the capillary wall as a major determinant of the transendothelial delivery of glucose [9] and insulin [10] in skeletal muscle since the interstitial concentrations of glucose and insulin are lower than those in the arterial plasma [9, 10]. This is further supported by the findings that the insulin- [11] and glucose- [12] stimulated increase in muscle blood flow correlates with glucose uptake in normal as well as in insulin resistant muscles [13].

The importance of blood flow for the rate of delivery of glucose and insulin to the muscles is, however,

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the subject of debate. Previous *in vivo* studies have shown that insulin increases blood flow in skeletal muscles both in humans [13] and in animals [14] and that the insulin resistance in muscle could be explained not only by defects in insulin action on muscle cells but also by a diminished vasodilatory effect of insulin in insulin-sensitive tissues [13]. When the extraction fraction of glucose was estimated concomitantly with blood flow in the muscle, however, only a small per cent of the increase in glucose uptake could be accounted for by the vasodilatory effect of insulin [15]. Others have reported that blood flow is not a major contributor to the uptake of glucose in individual muscles under basal conditions [16]. There is also direct evidence contrary to the hypothesis that blood flow is an independent regulator of insulin-stimulated glucose uptake in humans [17]. In view of the contradictory data, we studied the relation between muscle blood flow and metabolism during β -adrenergic stimulation, which is known to induce insulin resistance as well as peripheral vasodilation [1]. In a recent study on the human leg, the haemodynamic effects exerted by epinephrine were suggested to be an important compensating factor for the altered insulin sensitivity [18].

In this study, we used a previously described rat model [19] to investigate, *in situ*, the interactions between the microcirculation in the muscle and the delivery of glucose and insulin to the interstitial fluid. In the rat model, the effect of an infusion was compared to a previously measured control period in the same rat by the euglycaemic hyperinsulinaemic clamp technique. By combining microdialysis measurements in muscle interstitial fluid and recordings of muscle blood flow, the interaction between the microcirculation and the extraction of nutrients and hormones from the capillaries could be studied [20]. This approach allowed us to study the regulation of the interstitial fluid concentration of glucose and insulin and thus is probably more informative than the arterio-venous technique in describing the concentration-dependent extraction of these substances [9, 10]. Since their interstitial concentration is lower than in arterial plasma but higher than in the cells, two steps which limit the rate of muscle uptake of glucose and insulin should be considered; the plasma membrane and the capillaries. We tested the hypothesis that induction of insulin resistance and a shift in the rate limiting steps alters the interstitial fluid concentrations of glucose and insulin by studying the relation between the effects of acute β -adrenergic stimulation and β -adrenergic blockade on interstitial glucose and insulin concentrations as well as on blood flow. Microdialysis was performed in the medial femoral muscle during a hyperinsulinaemic euglycaemic clamp in rats.

Materials and methods

Female Sprague-Dawley (B & K Universal, Sollentuna, Sweden) rats weighing 277 ± 6 g were used. All rats were housed in groups of five rats per cage at 23°C and the dark-light cycle was 12:12 h. They were fed with commercial rat chow containing 18.5% protein, 4.0% fat and 55.7% carbohydrates with sufficient supply of vitamins and minerals (Lactamin, Stockholm, Sweden), and tap water *ad libitum*. The study was approved by the Animal Ethics Committee of Göteborg University.

Study procedure. In experiments to study the effect of epinephrine on interstitial glucose and lactate concentrations during hyperinsulinaemia, the euglycaemic clamp technique [21, 22] was used [23]. Briefly, the animals were anaesthetized with thiobutabarbital sodium, $170 \text{ mg} \cdot \text{kg}^{-1}$ body weight intraperitoneally (Inactin, RBI, Natick, USA), between 0800 and 0900 hours and placed on heating pads to maintain body temperature at 37°C . Catheters were inserted in the left carotid artery for blood sampling and in the right jugular vein for infusion of insulin and glucose. For measurements of heart rate, mean arterial blood pressure (MAP) and blood flow, a catheter was introduced in the caudal artery. Heart rate and arterial blood pressure were recorded every 15 min during the clamp.

After a 30 min postoperative period, a continuous infusion of $30 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ human insulin (Human Actrapid, 40 U/ml, Novo, Copenhagen, Denmark) started. Euglycaemia was maintained at 7 mmol/l plasma glucose concentration with 10% glucose solution in physiological saline. The infusion rate was guided by glucose concentration measurements in $30 \mu\text{l}$ blood samples at regular intervals (every 5 min during the first 40 min, then every 10 min throughout the clamp). Plasma lactate concentrations were measured concomitantly from the samples. Blood samples ($250 \mu\text{l}$) for analysis of plasma insulin were taken at 0, 40, 80, 120, 160, 200 and 280 min of the clamp. A total volume of less than 2 ml blood was used for these determinations.

In the insulin with epinephrine clamp plasma insulin steady state was reached approximately 40 min after initiation and kept for another 80 min before epinephrine (Sigma-Aldrich Chemical Co, St. Louis, Mo., USA) was infused at the rate of $0.15 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, together with isotonic saline and ascorbic acid (Apoteksbolaget, Göteborg, Sweden) (1.0 mg/ml) to prevent autooxidation [19]. The infusion was adjusted to achieve a sevenfold increase in plasma epinephrine compared with the control period with insulin infusion alone [19]. This epinephrine concentration has been shown to be similar to those observed during a bout of moderate exercise in the rat [19]. After 200 min, propranolol (Sigma-Aldrich Chemical Co.) ($2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was added to the insulin solution and infused to obtain total blockade of the β -adrenergic receptor [19]. We investigated 24 rats. Glucose infusion rate (GIR) was calculated as the mean of two determinations during the last 40 min in each infusion period and used as an index of insulin sensitivity.

Muscle blood flow in the probe membrane area and cardiac output were measured by the microsphere technique during insulin and insulin with epinephrine clamp [24]. In the additional experiments using the same protocol, the clamp was terminated after 80 min ($n = 8$) and after 160 min ($n = 8$), insulin and insulin with epinephrine, respectively, with an injection of microspheres according to the technique described below.

Another group of rats ($n = 8$) were treated in the same way with the addition of somatostatin (Sigma-Aldrich Chemical Co.) ($0.1 \mu\text{g/min}$) [25] infused with the insulin solution 10 min before the epinephrine infusion started to block the endoge-

nous insulin release. Somatostatin infusion continued until termination of the clamp at 280 min. In a further control study, the same protocol was used in combination with a higher insulin infusion rate to achieve maximum insulin stimulation ($240 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) ($n = 6$).

Microdialysis. Microdialysis was done in the medial femoral muscle in both legs, as described previously [20]. The two microdialysis catheters (OD 0.5 mm, length 15 mm, 40 kDa molecular weight cut-off; Bioanalytical Systems Inc., Indianapolis, Ind., USA) were connected to a microinjection pump (Carnegie Medicine, Stockholm, Sweden). They were perfused with 1% bovine albumin and [^3H]-glucose (Pharmacy, Sahlgrenska University Hospital, Göteborg, Sweden) in isotonic saline at a rate of $1 \mu\text{l}/\text{min}$. Starting 40 min after the insertion of the two catheters, 40 min samples of the interstitial fluid were collected for measurements of insulin, glucose and lactate during the clamp.

Calculations. The interstitial glucose concentration was calculated from the in vivo probe recovery according to the internal reference calibration technique [26]. The perfusate was prepared with [^3H]-glucose ($0.05 \mu\text{Ci}/\text{ml}$) and the loss over the membrane was taken as an estimate of recovery [26] during the periods of insulin infusion alone, epinephrine and propranolol period. The experiments showed a mean in vivo recovery of glucose during the three different periods of $55.4 \pm 5.8\%$ ($n = 22$). In experiments where the interstitial concentrations of insulin were estimated ($n = 68$), insulin was used as an external reference. [^{14}C]-insulin ($0.4 \text{ mCi}/\text{ml}$, New England Nuclear, Boston, Mass., USA) was infused subcutaneously ($8.0 \mu\text{l}/\text{h}$) for 24 h and in vitro experiments were done to correct for differences in binding and diffusion properties [27]. The in vivo relative recovery of insulin in microdialysate was $3.0 \pm 0.3\%$ [27]. The interstitial lactate concentration was calculated by dividing the dialysate lactate concentration by the empirical relative recovery factor 0.37, described previously from measurements in rat muscle under identical experimental conditions ($n = 20$, 0.37 ± 0.02) [27].

Measurement of blood flow. Skeletal blood flow was monitored using the microsphere technique [24]. The blood flow during insulin stimulation was measured by injection of radioactive [^{57}Co]-microspheres ($0.1 \text{ mCi}/\text{ml}$) in the left ventricle via the right carotid artery after 80 min of the clamp ($n = 8$). To explore the effect of acute β -adrenergic stimulation, the microspheres were injected after 160 min during insulin plus epinephrine in another group ($n = 8$). The injection was preceded by thorough mixing and ultrasonication of the microspheres and followed by a flush of 0.3 ml saline. The position of the heart catheter was verified as described previously [24]. A reference blood sample was drawn ($0.48 \text{ ml}/\text{min}$) from the caudal artery 2 min after the injection and while the rats were dying the muscles of the lower limb were excised. The blood flow rate in each muscle was calculated in $\text{ml} \cdot \text{min}^{-1} \cdot 100 \text{ g tissue}^{-1}$ and the cardiac output was calculated in ml/min [24] for the insulin and insulin plus epinephrine period.

Analytical methods. Blood was collected in heparinized microtubes and centrifuged immediately in a Beckman microfuge (Beckman, Palo Alto, Calif., USA). Plasma and microdialysate concentrations of glucose and lactate were determined enzymatically by using $10 \mu\text{l}$ samples for simultaneous analysis in a YSI 2700 select biochemical analyser (Yellow Springs Instrument Co. Inc. Yellow Springs, Ohio, USA). Plasma insulin was measured with a double antibody radioimmunoassay (Pharmacia, Uppsala, Sweden) and the insulin content in mi-

cro-dialysates was determined with an enzymatic immunoassay (DAKO Diagnostics Ltd, Cambridgeshire, UK) specific for human insulin, which is less efficient for rat insulin. Radioactivity in the microdialysate ([^3H]-glucose) was counted in a liquid scintillation counter using a quenched-corrected isotope program (1217 Rackbeta, LKB, Uppsala, Sweden) and in the muscles ([^{57}Co]-microspheres) with LKB Wallac 1282 Compu-gamma (Turku, Finland). Heart rate and mean arterial blood pressure (MAP) were displayed continuously by a multichannel recorder (Machine Technical Apartment, Sahlgrenska University Hospital, Göteborg, Sweden).

Statistical analyses. All results are expressed as means $\pm$ SEM. Statistical analyses were performed using analysis of variance (ANOVA) provided by the Stat-View program with the Macintosh system. Student's *t*-test for paired and unpaired observations was used for tests of significance of differences, *p* less than 0.05 being considered significant. Fisher's least significant difference test was used for post-hoc analyses.

Results

Basal conditions. Before the start of insulin infusion, the plasma insulin concentration was $174 \pm 18 \text{ pmol}/\text{l}$. The plasma glucose and lactate concentrations were $7.2 \pm 0.2 \text{ mmol}/\text{l}$ and $0.80 \pm 0.07 \text{ mmol}/\text{l}$ respectively. Heart rate was 358 ± 9 beats per min and the arterial blood pressure $115 \pm 6 \text{ mmHg}$.

The effect of insulin and β -adrenergic stimulation. The continuous insulin infusion during the clamp ($30 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) produced mean plasma insulin concentrations in the upper physiological range ($810 \pm 60 \text{ pmol}/\text{l}$) at 80 min of clamp, whereas the mean plasma glucose concentration was $7.6 \pm 0.2 \text{ mmol}/\text{l}$ during the same period (Fig. 1). Epinephrine infusion and associated β -adrenergic stimulation increased plasma insulin concentration from 810 ± 60 to $1026 \pm 84 \text{ pmol}/\text{l}$ ($p < 0.05$) but the interstitial insulin concentration remained constant (666 ± 42 vs $754 \pm 42 \text{ pmol}/\text{l}$, NS, Fig. 1).

The epinephrine infusion induced a pronounced (72%) reduction of glucose infusion rate compared with insulin infusion alone (10.1 ± 1.14 vs $3.7 \pm 0.59 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p < 0.0001$, Fig. 2). Concomitantly, the interstitial glucose concentration increased (Table 1). The glucose infusion rate and interstitial glucose concentration during epinephrine infusion correlated negatively ($n = 22$, $r = 0.51$, $p < 0.0001$). The arterial-interstitial concentration difference of glucose was decreased by epinephrine from 2.52 ± 0.32 (at 40–120 min) to 1.20 ± 0.43 (at 120–200 min) mmol/l ($p < 0.05$, Table 2).

The lactate concentration in plasma as well as in interstitial fluid (Table 1) was increased by epinephrine. The interstitial-arterial concentration difference of lactate was also increased, 1.12 ± 0.15 vs $1.47 \pm 0.32 \text{ mmol}/\text{l}$, $p < 0.05$ (Table 2). Heart rate tended to increase (329 ± 8 vs 343 ± 11 beats per min, NS) and

Fig. 1 A, B. Effects of epinephrine and propranolol on the concentration of insulin in plasma ($n = 22$) (□) and the interstitial fluid ($n = 22$) (■) during an euglycaemic hyperinsulinaemic clamp ($30 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) alone (**A**) or in combination with somatostatin infusion ($n = 8$ for plasma and interstitial fluid) (**B**). Depicted plasma insulin concentrations were determined from mean values of plasma samples taken after 40, 80, 120, 160, 200, 240 and 280 min of clamp. Interstitial insulin concentrations were determined from the pooled microdialysates ($2 \times 40 \text{ min}$) collected during each infusion period. * $p < 0.05$ in plasma insulin concentration between insulin + epinephrine vs insulin and insulin + epinephrine + propranolol. Data are means $\pm$ SEM

Table 1. The effect of epinephrine and propranolol on the glucose and lactate concentration in plasma and in the interstitial fluid during an euglycaemic hyperinsulinaemic clamp (insulin infusion rate $30 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Data obtained at state conditions achieved after 40 min and given as means $\pm$ SEM

	insulin	+ Epi	+ Epi + Prop
Plasma-glucose	7.5 ± 0.2	7.9 ± 0.3	7.1 ± 0.2
Interstitial-glucose	5.0 ± 0.2^c	6.7 ± 0.5^a	4.6 ± 0.3^c
Plasma-lactate	1.4 ± 0.1	2.7 ± 0.4^b	1.5 ± 0.3
Interstitial-lactate	2.5 ± 0.2^c	$4.1 \pm 0.6^{a,c}$	2.8 ± 0.4^c

^a $p < 0.01$, ^b $p < 0.001$, insulin + epinephrine vs insulin and insulin + epinephrine + propranolol

^c $p < 0.05$, interstitial fluid vs plasma

Fig. 2. Effects of epinephrine and propranolol on mean glucose infusion rate, ($n = 22$) during an euglycaemic hyperinsulinaemic clamp (insulin infusion $30 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Data are means $\pm$ SEM, *** $p < 0.001$ insulin + epinephrine vs insulin and insulin + epinephrine + propranolol

the arterial blood pressure was maintained after epinephrine (93 ± 4 vs 82 ± 7 mmHg, NS).

The effect of insulin, epinephrine and propranolol. Propranolol reversed the epinephrine-induced increase of the plasma insulin concentration whereas no significant change in the interstitial insulin concentration was detected (Fig. 1). The reduction in glucose infusion rate induced by epinephrine was also reversed by addition of propranolol when it increased from 2.8 ± 0.59 to $10.7 \pm 1.14 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ($p < 0.0001$, Fig. 2). Concomitantly, the interstitial glucose concentration decreased below the basal level (Table 1). The arterial-interstitial concentration difference of glucose was increased by propranolol (Table 2). The plasma lactate concentration was restored to the pre-epinephrine state during propranolol infusion as was the interstitial lactate concentration (Table 1). The interstitial-arterial concentration difference of lactate decreased (Table 2). After propranolol was given there was a diminution of the heart rate (343 ± 11 vs 307 ± 12 beats per min, $p < 0.05$) and of the arterial blood pressure (82 ± 7 vs 60 ± 9 mmHg, $p < 0.05$).

The effect of epinephrine and propranolol during somatostatin and insulin infusion. To further examine whether the effect of epinephrine on the insulin concentration in plasma was due to enhancement of insulin secretion or altered distribution volumes, additional experiments were done with somatostatin. The infusion of somatostatin totally abolished the epinephrine-induced increase in plasma insulin concentration whereas the interstitial insulin concentration remained unchanged (Fig. 1).

In further experiments the insulin infusion was increased to maximum levels ($240 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)

Table 2. The arterial-interstitial concentration difference of glucose ($\text{mmol} \cdot \text{l}^{-1}$) and lactate ($\text{mmol} \cdot \text{l}^{-1}$) during an euglycaemic hyperinsulinaemic clamp (insulin infusion $30 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), with or without infusions of epinephrine and propranolol

	Arterial-interstitial concentration difference	
	Glucose ($\text{mmol} \cdot \text{l}^{-1}$)	Lactate ($\text{mmol} \cdot \text{l}^{-1}$)
Insulin	2.52 ± 0.32	(-) 1.12 ± 0.15
Insulin + epinephrine	$1.20 \pm 0.43^*$	(-) $1.47 \pm 0.32^*$
Insulin + epinephrine + Propranolol	2.50 ± 0.26	(-) 1.18 ± 0.31

Data are means $\pm$ SEM and given as averages during each period (insulin and insulin + epinephrine), $n = 22$. * $p < 0.05$ compared with insulin and insulin + epinephrine + propranolol

to completely block the endogenous insulin secretion. Under these circumstances, neither epinephrine nor somatostatin altered the insulin concentration either in plasma or in interstitial fluid (not shown).

Blood flow measurements. Leg muscle blood flow was examined by the microsphere technique during submaximum insulin infusion ($30 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Mean muscle blood flow was greatly enhanced (133%) with epinephrine (Table 3). An increase in blood flow was seen in all muscles, except in the Soleus muscle. Cardiac output tended to increase during epinephrine infusion without reaching statistical significance (Table 3).

Discussion

This study confirms previous findings that glucose [9] and insulin [10] concentrations are lower in the muscle interstitial fluid than in arterial plasma. The data show for the first time that muscle insulin resistance induced by β -adrenergic stimulation leads to increased interstitial fluid concentrations of glucose and lactate and vasodilatation without altering the interstitial concentration of insulin. The increased in-

terstitial glucose concentration showed similar time kinetics as the induction of insulin resistance and is the result of decreased cellular uptake of glucose together with increased delivery of glucose by vasodilatation. This also shows that the cellular resistance to insulin induced by epinephrine and β -adrenergic stimulation cannot be overcome by either increased secretion of insulin or vasodilation, and, moreover, casts doubt on the view that vasodilatory agents might reverse severe insulin resistance. Since the transport of glucose is a facilitated diffusion process, it cannot, however, be excluded that insulin-independent glucose uptake might be enhanced by vasodilation and increase of the interstitial glucose. In a study on the human leg [18], it was suggested that vasodilation induced by moderately high doses of epinephrine in the presence of supraphysiological concentrations of insulin might totally compensate for the inhibition of glucose extraction by the muscle cells. The data were derived, however, from two small subject groups given either epinephrine or saline [18]. Our data, obtained in experiments where each animal serves as its own control, suggest that compensation in terms of higher interstitial concentrations of glucose might already occur at physiological insulin concentrations but that this is not enough to overcome the reduction in glucose uptake.

The microdialysis technique used here was validated recently in the rat muscle and was shown not to alter either the capillary permeability or interstitial fluid concentration of either glucose or insulin [26, 27]. In our study (data not shown), the constant dialysis recovery of inulin and retrodialysis of labelled glucose ascertained constant and steady state microdialysis conditions throughout the study. It can thus be concluded that our results were not affected by artifacts created by the microdialysis technique.

By combining the microdialysis technique with blood flow measurements, the extraction fraction nutrients and hormones from the plasma compartments could be calculated by means of Fick's principle [29]. The extraction fraction can be calculated according to the formula $EF = (A-I) \cdot (1 - e^{-PS/Q})$, where EF is the extraction fraction, (A-I) is the arterial-interstitial

Table 3. Cardiac output (ml/min) and skeletal muscle blood flow ($\text{ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$) during an euglycaemic hyperinsulinaemic clamp (insulin infusion rate $30 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) with or without additional infusion of epinephrine ($2.0 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)

	Cardiac output ($\text{ml} \cdot \text{min}^{-1}$)	Blood flow							
		Tibialis anterior	EDL	Gastrocnemius white	Gastrocnemius red ($\text{ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$)	Plantaris	Soleus	Femoral medial	Average
Insulin	39 ± 5	39 ± 6	25 ± 3	28 ± 5	40 ± 6	35 ± 5	49 ± 6	35 ± 6	36 ± 2
Insulin + epinephrine	58 ± 7	$140 \pm 22^{***}$	$81 \pm 14^{***}$	$79 \pm 11^{***}$	$63 \pm 9^*$	$85 \pm 14^{**}$	50 ± 8	$113 \pm 21^{**}$	$84 \pm 6^{***}$

Data are means $\pm$ SEM, $n = 8$. Extensor digitorum longus (EDL) * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ insulin vs, insulin + epinephrine

concentration difference, PS is the permeability surface area product and Q is the plasma flow. The present A-I concentration difference of glucose and the assumption that $PS = 19 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ [29] when the blood flow is $36 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ gives an estimated glucose uptake of approximately $28 \text{ } \mu\text{mol} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ during insulin infusion alone. During epinephrine infusion the permeability surface area product could be expected to be approximately $24 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ [29] and muscle glucose uptake could then be estimated to be approximately $21 \text{ } \mu\text{mol} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$, which represents about 24% decrease. The blood flow data obtained in our study agree with those measured in rats during slow treadmill exercise, using the microsphere technique [24, 30]. Concomitantly, other factors than reduced glucose uptake in the muscle, such as liver glucose output, contribute to the decrease in glucose infusion rate during epinephrine and β -adrenergic stimulation. Previous findings in hypoglycaemic rats suggest a similar decrease in peripheral glucose uptake due to increased sympathetic nervous activity and β -adrenergic stimulation [31]. Furthermore, studies in man have shown similar magnitudes of insulin resistance after epinephrine exposure [32]. This, and the protocol we used allows the maintenance of epinephrine concentrations in the upper physiological range [19], indicates that our data are physiologically relevant and, further, suggests that acute insulin resistance can be accomplished by increased interstitial glucose concentrations. It could be possible that this, in turn, might induce insulin resistance even under normoglycaemic conditions through glucose toxicity [33]. Insulin resistance could be induced by high glucose concentrations ambient to the cells through formation of hexosamines, interfering directly with the insulin signal transduction. Also, it is notable that the insulin-resistant Zucker rat has higher than normal glucose concentrations in muscle interstitial fluid even under normoglycaemic insulin clamping conditions [27].

In contrast to the decrease in A-I concentration difference for glucose, the lactate I-A concentration difference increased during epinephrine exposure. When the above formula was applied, lactate production was estimated to be approximately $14 \text{ } \mu\text{mol} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ during exposure to insulin alone and approximately $28 \text{ } \mu\text{mol} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ during epinephrine infusion. Hence, about 100% increase in lactate release was shown, suggesting a considerable shift from oxidative to non-oxidative glycolysis during β -adrenergic stimulation presumably by activation of glycogenolysis. This agrees with previous studies in vivo [7] and in vitro [34] showing that, despite the reduced glucose uptake lactate production is favoured after β -adrenergic stimulation.

Studies in lymph [35] as well as in interstitial fluid [10] by means of microdialysis have shown that the interstitial insulin concentration is about 50% lower

than in arterial plasma. In rat muscle, however, a clear difference between interstitial muscle insulin and plasma insulin is evident only at concentrations in the upper physiological range, that is beyond approximately 1.5 nmol/l , which in our clamp model equals the EC_{50} for the effect of insulin on glucose infusion rate [36]. The present data confirm that the interstitial insulin concentration is similar to plasma insulin in the lower concentration range. Epinephrine infusion rapidly increased plasma insulin and in control experiments where insulin secretion was blocked by either supraphysiological concentrations of insulin or somatostatin the epinephrine effect was abolished. These results are compatible with recent data showing that epinephrine induces peripheral insulin resistance as well as increases insulin release [37]. The increase in plasma insulin we found was not followed by an increase in interstitial insulin. It should be noted that the interstitial insulin concentration and the content of insulin in plasma were analysed by two different immunoassay techniques and that endogenous rat insulin is less recognized efficiently by the method of analysis used for the microdialysates. Hence, it cannot be excluded that the nonsignificant increase in interstitial insulin during epinephrine exposure has been underestimated. Also, a less pronounced increase in interstitial insulin could be due to the time delay in changes of the insulin concentration in the interstitial fluid reported previously [10] as well as the increase in difference between the interstitial and plasma concentrations that appears in the higher concentration range even at steady state [10]. It is notable that a saturable transport route for insulin [10], through the receptor-related trans-endocytosis pathway, has been proposed [38]. To further elucidate the tentative effects of epinephrine on insulin distribution and compartmentalization, additional experiments were done, where insulin secretion was blocked either by somatostatin or by supraphysiological insulin concentrations. The data from these experiments show no changes in either plasma or interstitial insulin during exposure to epinephrine. This might provide indirect evidence of the existence of an alternative route for insulin transport since vasodilation induced by epinephrine resulted in increased glucose, but not insulin, concentrations in the interstitial fluid. The data do not enable us to conclude whether this was due to a decreased delivery of insulin to the interstitial fluid or to an increase of the cellular insulin uptake. The second explanation seems likely, however, since epinephrine and β -adrenergic stimulation rapidly decrease insulin binding as well as the receptor number in the plasma membrane of adipose and muscle cells [3, 4]. Furthermore, preliminary data from cultured endothelial cells indicate that insulin receptor binding in this cell type is diminished rapidly by β -adrenergic stimulation (unpublished observation).

In summary, insulin resistance and vasodilation in the muscle induced by β -adrenergic stimulation are followed by increased interstitial concentrations of glucose and lactate without alterations of interstitial insulin despite an increased insulin secretion rate. The data suggest that an increased delivery rate of glucose and insulin is likely to prevent severe insulin resistance.

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