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Membrane transport in relation to net uptake of glucose in the perfused rat hindlimb

Stimulatory effect of insulin, hypoxia and contractile activity

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1. The paired-tracer dilution method applied to the perfused rat hindlimb model was used to study glucose transport in relation to net glucose uptake in skeletal muscle tissue. 2. 2-deoxyglucose was used as an analogue for glucose, since this eliminates the problem with release of labelled metabolites. The affinity of 2-deoxyglucose for the glucose carrier was shown to be indistinguishable from that of glucose. 3. An insulin dose–response study showed maximal stimulation of glucose uptake and transport at 0.1 unit/l, and 75% of maximal stimulation at 0.01 unit of insulin/l. 4. Hypoxia and contractile activity stimulated the 2-deoxyglucose transport rate similarly, and the stimuli were not additive, suggesting a common mechanism. 5. The presence of insulin did not increase the effect of hypoxia or contractile activity, indicating no permissive effect of insulin. 6. The 2-deoxyglucose transport rate was closely correlated with and always higher than that of glucose uptake, demonstrating that the transport is never rate-limiting for the net glucose uptake and that both processes are regulated together. 7. Significant correlations between the 2-deoxyglucose transport rate and the intramuscular concentration of phosphocreatine suggest regulation of the glucose utilization by the energy state of the skeletal muscle tissue.

INTRODUCTION

Glucose uptake is an important process in the fuel economy of skeletal muscle and is known to be under the modulation of a variety of physiological stimuli such as those provided by insulin, hypoxia and contractile activity (Randle & Smith, 1958; Morgan *et al.*, 1961; Szabo *et al.*, 1969; Berger *et al.*, 1975). The mechanisms involved remain largely unknown, but most involve each of three processes, the net result of which is glucose uptake. These processes are membrane transport, post-membrane phosphorylation of glucose by hexokinase and subsequent removal of glucose 6-phosphate by the routes of glycolysis and glycogen synthesis. It is traditionally thought that the process of membrane transport is rate-limiting for glucose uptake and, since glucose has been rarely measured as accumulating in the intracellular space, the post-membrane metabolic events have no influence on the net flux of glucose into muscle (Berger *et al.*, 1975; Goodman & Ruderman, 1979; Richter *et al.*, 1982). Although such a view is consistent with much of the information available to date, much of the evidence is indirect and not derived from specific measurements of glucose transport itself and comparisons with net uptake.

We have used a recently developed technique for the measurement of the unidirectional membrane transport of glucose in the perfused rat hindlimb (Rennie *et al.*, 1983) to re-investigate the relationship between the glucose transport and the net uptake of glucose, measured by means of the arterio-venous difference. In particular we have investigated the possibility that the stimulatory effects exhibited by insulin, hypoxia and contractile activity are exerted by a common mechanism. A preliminary communication of parts of this work has been presented (Bylund-Fellenius *et al.*, 1982).

MATERIALS AND METHODS

Chemicals

Substrates, cofactors and enzymes were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Bovine serum albumin (fraction V) was obtained from Miles Laboratories (Axel Johnson and Co., Malmö, Sweden). [^{14}C]Mannitol and 2-deoxy[^{3}H]glucose were purchased from New England Nuclear (Düsseldorf, Germany).

Analyses

Glucose was determined by means of a glucose GOD-Perid kit from Boehringer, Mannheim, Germany. Lactate was separated by t.l.c. on silica gel 60 (Merck, Darmstadt, Germany). The muscle concentrations of phosphocreatine (PCr) and glycogen were determined respectively by the methods described by Bylund-Fellenius *et al.* (1982) and Hultman (1967). Insulin was analysed by radioimmunoassay (Pharmacia Diagnostics, Uppsala, Sweden).

Animals

Female Sprague–Dawley rats weighing 230–270 g were used. The rats were fed on Purina chow and water *ad libitum*, and were anaesthetized with an intraperitoneal

Abbreviation used: PCr, phosphocreatine.
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injection of Mebumal (ACO, Solna, Sweden) (30 mg/kg body wt.).

Rat hindlimb perfusion

Rats were prepared for perfusion of one hindlimb by the technique described previously (Walker *et al.*, 1982; Idström *et al.*, 1985). The hindlimbs were perfused with Krebs–Henseleit bicarbonate buffer, pH 7.4, containing 6% (w/v) bovine albumin, at a flow rate of 0.33 ml/min per g of muscle. For normoxic conditions the perfusate was gassed with O_2/CO_2 (19:1), giving perfusate pO_2 of approx. 60 kPa. When hypoxic conditions were to be studied, an N_2/CO_2 (19:1) gas mixture was used, giving perfusate pO_2 of approx. 6 kPa. The perfusate glucose concentration was in the range 1–50 mM, and that of insulin 0.01–1.0 unit/l.

Contractile activity was induced by direct stimulation of the muscle of the skinned hindlimb with aluminium-foil electrodes moistened with 0.9% NaCl or by indirect stimulation via the sciatic nerve. The resting tension and the voltage were adjusted to obtain optimal contractions, as judged from the isometric tension measured with a strain gauge. Tetanic contractions of 100 ms duration, at 60 Hz, were induced once a second with an electric stimulator (Grass SD-5).

Paired-tracer dilution method

Measurements of unidirectional transport of 2-deoxyglucose were carried out by means of the paired-tracer dilution method (Yudilevich & Mann, 1982), as applied to the perfused rat hindlimb (Rennie *et al.*, 1983). In brief, this technique involves arterial injection of a mixture of an extracellular reference tracer, [^{14}C]mannitol, and a glucose analogue, 2-deoxy[^{3}H]-glucose, the transport of which is to be studied. The method relies on the differential passage into the tissue of the substance of interest in relation to the extracellular tracer. The venous effluent is collected in fractions over a 2 min period after the injection. The transport rate is assessed from the amounts of ^{14}C and ^{3}H present in the venous samples in relation to the injected dose.

We chose to use 2-deoxyglucose as a glucose tracer for the transport-rate measurements, since it is not metabolized beyond the phosphorylation step. The trapping of the tracer within the tissue allows calculation of the undirectional influx of glucose, without interference from the appearance of labelled glucose metabolites in the venous effluent.

Experimental procedure

All perfusions began with a 20 min equilibration period at normoxia, i.e. with an O_2 delivery of 10–15 μmol/h per g of muscle. Thereafter, the first transport measurement was performed by injection and venous collection over a 2 min period. An estimate of total recovery of the injected tracer was obtained by an additional 2 min collection. The system was then altered to investigate the transport under a different condition. The hindlimb was equilibrated to the new condition for 10 min before the next transport measurement was carried out. At rest, conditions could be changed eight times without any signs of degeneration of the preparation in terms of O_2 consumption, arterial pressure and flow rate. Muscle stimulation was always applied as the last procedure. The net glucose uptake was calculated for each condition from glucose analysis of arterial and venous perfusate samples taken after the transport measurement. Flow was measured by timed collection and weighing of the venous effluent.

A prerequisite for an accurate determination of glucose transport is that the affinity of the glucose carrier for 2-deoxyglucose is similar to that of glucose. This was investigated by arterial injection of [^{14}C]glucose and 2-deoxy[^{3}H]glucose in a medium containing 10 mM-glucose, into hindlimbs equilibrated with perfusate containing 10 mM-glucose (normoxia; insulin, 0.1 unit/l). The venous effluent was collected in fractions and the ^{3}H and ^{14}C radioactivities were counted. The venous fractions were also used for determination of ^{14}C labelling appearing as lactate. The lactate was separated by t.l.c., and the radioactivity obtained in the lactate spot was expressed in relation to the total ^{14}C radioactivity in the corresponding venous sample. The total proportion of the radioactivity appearing as CO_2 in the venous effluent was determined by difference after acidifying and heating a sample of the perfusate. In six experiments the $^{14}CO_2$ produced accounted for only 2.48 ± 0.93% (mean $\pm$ S.E.M.).

A transport-index (T-index) was calculated to evaluate the difference in transport characteristics between glucose and 2-deoxyglucose. The mathematical expression for the T-index is [1 – (^{3}H % of dose/^{14}C % of dose)] + 1, where the radioactivity percentage of dose is the radioactivity in the venous effluent in relation to the injected dose. The deviation of the T-index from 1.0 provides an estimate of the apparent difference in transport between the two analogues.

In another series of experiments, arterial injection of 2-deoxy[^{3}H]glucose and [^{14}C]glucose in a medium containing 2, 10 or 25 mM unlabelled glucose was performed into hindlimbs equilibrated with perfusate containing no glucose (normoxia; insulin, 0.1 unit/l). The T-index 1 min after injection was calculated as described above, to estimate the competition for the glucose carrier at various glucose concentrations.

The effect of insulin on the 2-deoxyglucose transport, as well as on the net glucose uptake, was evaluated. The experiments were carried out under normoxic conditions with a perfusate glucose concentration of 10 mM, and insulin concentrations between 0 and 1.0 unit/l. Samples of the perfusate were taken for determination of the insulin concentration.

The effect of hypoxia on 2-deoxyglucose transport and net glucose uptake was evaluated at glucose concentrations of 1–50 mM in the absence and in the presence of insulin (0.1 unit/l). In these experiments, the O_2 delivery was 1–2 μmol/h per g of muscle. For comparison, rats were also perfused under normoxic conditions with and without insulin. At the end of the experiments, biopsies were taken from the soleus and the superficial part of the lateral gastrocnemius muscle. The biopsies were rapidly frozen in liquid N_2 and analysed for PCr and glycogen.

The 2-deoxyglucose transport rate was determined during contractile activity under normoxic conditions, at perfusate glucose concentrations of 2, 10 and 25 mM, in the absence and in the presence of insulin (0.1 unit/l). Further experiments were carried out to study the effect of contractile activity under hypoxic conditions in the presence of insulin (0.1 unit/l), at 2 mM-, 10 mM- and 25 mM-glucose in the perfusate. Muscle samples for metabolite analysis were obtained at the end of the experiments, as described above.

RESULTS

Glucose and 2-deoxyglucose transport

The possible difference in the affinity of the carrier for glucose and 2-deoxyglucose was assessed by injecting labelled glucose (^{14}C) and labelled 2-deoxyglucose (^{3}H) simultaneously. The time course of the changes in the T-index in the venous-effluent fractions collected after injection of the labelled sugars is shown in Fig. 1 (●). The T-index was 1.061 ± 0.009 during the first minute and increased during the second minute in an exponential fashion. At 2 min after the injection the T-index was 1.187 ± 0.026.

The conversion of the injected [^{14}C]glucose into [^{14}C]lactate was measured by t.l.c. separation of the venous samples. The ratio between the [^{14}C]lactate and the total ^{14}C in the corresponding fraction is also shown in Fig. 1 (○). The release of [^{14}C]lactate increased exponentially with time. This release of ^{14}C label in the form of lactate will give an underestimation of the transport rate for [^{14}C]glucose. Thus the T-index must be corrected for the amount of label released as [^{14}C]lactate. The corrected T-index as a function of time showed that within the 2 min period the ratio fluctuated between 1.030 ± 0.011 and 0.960 ± 0.016 (mean ± S.E.M.). The interference with the T-index by the evolution of $^{14}CO_2$ was less than 3%, which is within the error of the ratio as measured here.

The T-index measured 1 min after the injection at 2 mM-, 10 mM- and 25 mM-glucose was 1.10 ± 0.04, 1.08 ± 0.10 and 1.03 ± 0.07 respectively ($n = 3$).

Insulin dose–response relationship

The stimulatory effect of insulin was assessed at a glucose perfusate concentration of 10 mM under normoxic conditions. The 2-deoxyglucose transport rate as well as the net glucose uptake were determined at different insulin concentrations (0.01–1.0 unit/l) and the dose–response curves were plotted (Fig. 2). A 75% stimulation of both transport and net uptake was observed at the lowest concentration studied (0.01 unit/l). Maximal

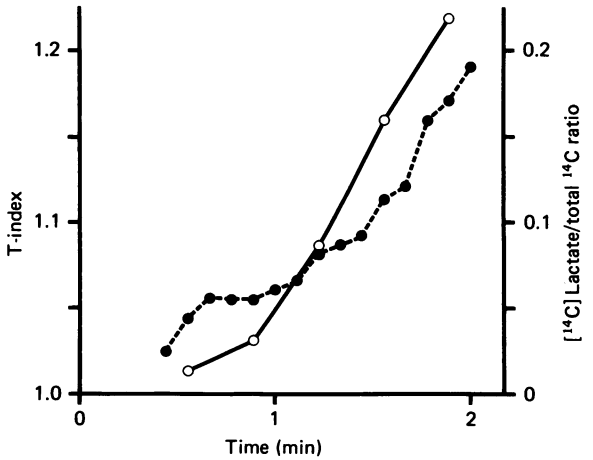

Fig. 1. Changes in the transport rate index (T-index) (●) and the [^{14}C]lactate/total ^{14}C ratio (○) after injection of 2-deoxy[^{3}H]glucose and [^{14}C]glucose into the perfused rat hindlimb at rest

For experimental details see the Materials and methods section. Results are means ± S.E.M. ($n = 6$).

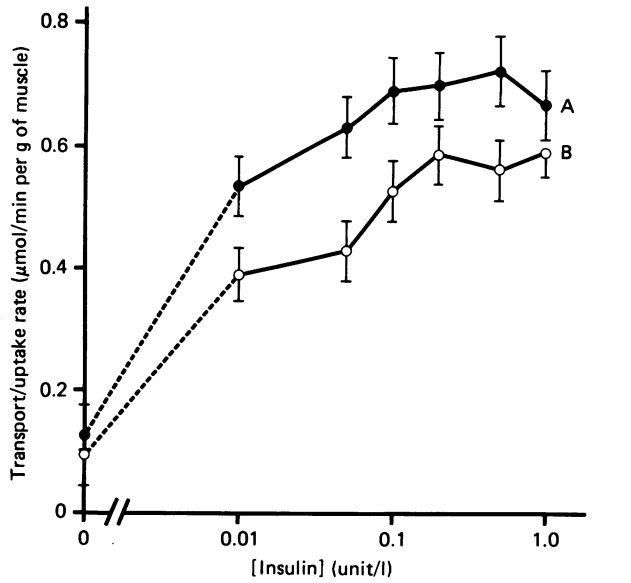

Fig. 2. Insulin dose–response curve in the perfused rat hindlimb at rest under normoxic conditions at a perfusate glucose concentration of 10 mM

A full range of insulin concentrations were investigated in each rat. Curve A, glucose transport rate; curve B, glucose uptake. Results are means ± S.E.M. ($n = 8$).

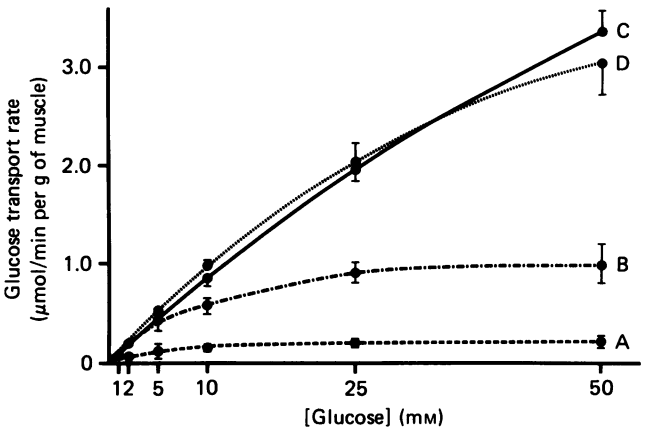

Fig. 3. Effect of perfusate glucose concentration on the transport rate of glucose in the perfused rat hindlimb at rest

Curve A, normoxia, no insulin; curve B, normoxia, 0.01 unit of insulin/l; curve C, hypoxia, no insulin; curve D, hypoxia, 0.1 unit of insulin/l. Results are means ± S.E.M. ($n = 6$–13).

2-deoxyglucose transport and net glucose uptake were achieved at 0.1 unit of insulin/l. The transport rate was higher than the net uptake at all insulin concentrations.

Effect of hypoxia

The 2-deoxyglucose transport rate was stimulated by hypoxia, and identical rates were obtained in the absence and in the presence of insulin (0.1 unit/l) (Fig. 3). The stimulatory effect of hypoxia on the 2-deoxyglucose transport rate increased with the glucose concentration (Fig. 3 and Table 1). In the absence of insulin the transport rate was stimulated by hypoxia over the whole

Table 1. 2-Deoxyglucose transport rates at glucose perfusate concentrations of 2 and 25 mM under various conditions

For details see the Materials and methods section. Results are means ± S.E.M. for the numbers of experiments in parentheses, and are expressed as μmol/min per g of muscle.

	[Glucose]	Rest	Rest, hypoxia	Contracted	Contracted, hypoxia
No insulin	2 mM	0.041 ± 0.006 (6)	0.194 ± 0.020 (6)	0.173 ± 0.015 (3)	—
	25 mM	0.180 ± 0.017 (6)	1.915 ± 0.017 (8)	2.050 ± 0.040 (3)	—
Insulin (0.1 unit/l)	2 mM	0.240 ± 0.045 (13)	0.220 ± 0.031 (7)	0.170 ± 0.022 (9)	0.220 ± 0.019 (5)
	25 mM	0.900 ± 0.103 (13)	2.160 ± 0.172 (9)	1.620 ± 0.190 (10)	1.950 ± 0.310 (5)

range of glucose concentrations studied. In the presence of insulin the transport rate was significantly stimulated by hypoxia at glucose concentrations above 10 mM, but not at 5 mM and below. At 50 mM-glucose, hypoxia caused a 3-fold increase in the transport rate in the presence, and a 17-fold increase in the absence, of insulin, respectively (Fig. 3).

Since no saturation was obtained in the 2-deoxyglucose transport rates during hypoxia, no reliable kinetic constants could be calculated. From the transport kinetic curves obtained for rats perfused at normoxia, a K_m of 9.4 ± 2.5 mM and a $V_{max.}$ of 1.10 ± 0.03 μmol/min per g of muscle in the presence of insulin and a K_m of 10.5 ± 0.9 mM and a $V_{max.}$ of 0.25 ± 0.01 μmol/min per g of muscle in the absence of insulin could be computed by the method of Atkins & Gardner (1977).

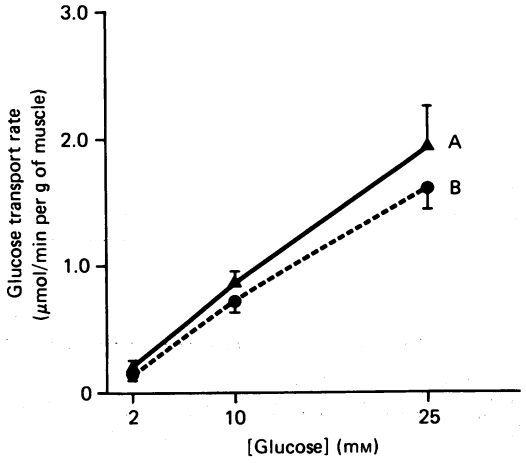

Fig. 4. Effect of perfusate glucose concentration on the transport rate of glucose in the perfused contracting rat hindlimb, in the presence of insulin (0.1 unit/l)

Curve A, hypoxia; curve B, normoxia. Results are means ± S.E.M. (n = 5–10).

Effect of contractile activity

The effect of tetanic contractions on the 2-deoxyglucose transport rate was investigated at perfusate glucose concentrations of 2 mM, 10 mM and 25 mM in the absence and in the presence of insulin (0.1 unit/l), in the latter case, under both normoxic and hypoxic conditions (Table 1 and Fig. 4).

Under normoxic conditions, contractile activity stimulated the transport rate by 0, 50 and 100% at 2 mM-, 10 mM- and 25 mM-glucose respectively. The transport rate achieved during contractile activity was similar both in the presence and in the absence of insulin.

Although hypoxia alone caused a considerable augmentation of the resting glucose transport, hypoxia during contractile activity did not cause a further stimulation of the transport rate, no matter in which order contractions and hypoxia were induced. Thus the transport rate during contractile activity in combination with hypoxia was similar to that obtained when hypoxia and contractions were applied separately (Table 1).

Transport versus net uptake of glucose

In parallel with the transport-rate measurements, the net glucose uptake was determined at rest (with or without insulin) and at rest with hypoxia (with or without insulin). Under these conditions the net glucose uptake, as well as the transport rate, varied over a broad range (0.11–3.25 μmol/min per g of muscle). As illustrated in Fig. 5, there was a close correlation between the transport rate and the net uptake of glucose (r = 0.99, P < 0.001) under these conditions. Furthermore, the transport rate was higher than the net uptake by approx. 20%.

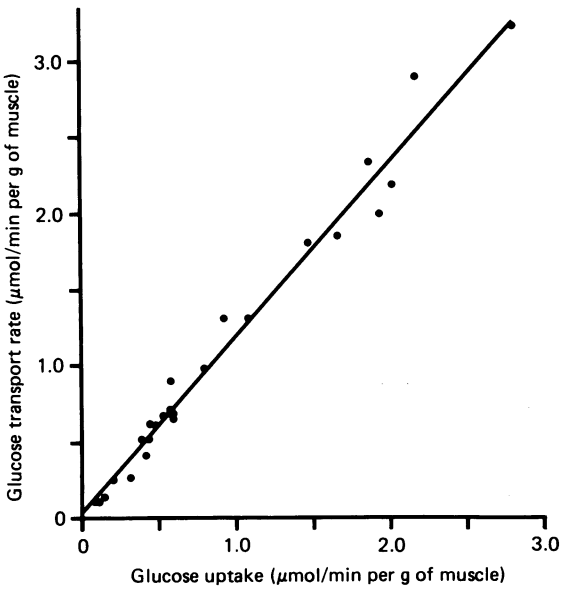

Fig. 5. Relation between glucose uptake and glucose transport rate in the perfused rat hindlimb

For experimental details see the Materials and methods section. The line of best fit to the data is $y = 1.17x + 0.004$; r = 0.99, P < 0.001, n = 26.

Table 2. Concentrations of phosphocreatine and glycogen in the soleus and gastrocnemius muscles of the perfused rat hindlimb at different perfusate glucose concentrations under various conditions

For experimental details see the Materials and methods section. Results are means ± S.E.M. for the numbers of experiments in parentheses.

		Concn. (μmol/g dry wt.)			
		Rest	Contraction and hypoxia		
	Muscle [Glucose] (mM)...	10	2	10	25
PCr	Soleus	70.8±8.3 (6)	16.7±7.1 (8)	12.5±9.9 (8)	12.4±8.6 (9)
	Gastrocnemius	96.2±9.5 (6)	26.6±9.2 (6)	29.4±7.4 (11)	27.8±6.4 (10)
Glycogen	Soleus	147*±12 (8)	55.5±7.1 (8)	67.9±7.0 (10)	72.6±7.2 (10)
	Gastrocnemius	145*±10 (8)	48.7±6.4 (8)	67.8±7.4 (13)	80.0±7.1 (10)

* Values obtained from normal resting non-perfused rat hindlimbs.

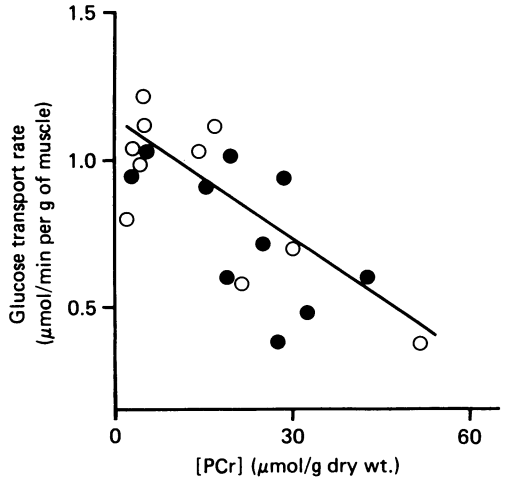

Fig. 6. Relation between the intramuscular concentration of phosphocreatine and the glucose transport rate at the end of a period of hypoxia and/or contractile activity in the perfused rat hindlimb at 10 mM perfusate glucose concentration

The line of best fit to the data is $y = -0.013x + 1.12$; $r = -0.72$, $P < 0.001$, $n = 20$. For experimental details see the Materials and methods section (○, soleus; ●, gastrocnemius).

Muscle metabolites

Muscle samples for metabolite analysis were taken at the end of all experiments performed under conditions likely to cause an alteration of the energy state (i.e. contractile activity and hypoxia). The concentrations of PCr and glycogen in the soleus and gastrocnemius muscles at different perfusate glucose concentrations are shown in Table 2. The lower the glucose concentration of the perfusate, the lower the glycogen content in both muscles, whereas such a relationship did not exist for PCr. A net glycogen breakdown at all three glucose concentrations was observed, since the normal resting glycogen concentration in gastrocnemius and soleus muscle is approx. 140 μmol/g dry wt. Thus the dependence on glycogen as substrate was greater the lower the availability of exogenous glucose during hypoxia and/or contractile activity.

A relationship between the PCr concentration and the 2-deoxyglucose transport rate was obtained in both the soleus and the gastrocnemius muscles at 10 mM-glucose (Fig. 6). The correlations were identical for both muscles, and therefore the data for both muscles were evaluated together. The corresponding relation did not exist at 2 mM- or at 25 mM-glucose.

DISCUSSION

2-Deoxyglucose has previously been used as an analogue for glucose in membrane transport studies (Kipnis & Cori, 1959; Morgan & Whitfield, 1974). We could here demonstrate that there is no difference in transport between glucose and 2-deoxyglucose, since they are transported identically when the release of label from metabolized glucose is taken into account. Thus, within 1 min after the injection of 2-deoxy[^{3}H]glucose and [^{14}C]glucose, the variation of the T-index was less than ±8%. Owing to the time delay for metabolizing the [^{14}C]glucose to [^{14}C]lactate, the T-index fluctuated during the measuring period. The presence of significant amounts of ^{14}C label in a major metabolite such as lactate within 2 min after the injection confirms that 2-deoxyglucose is a better choice for our purpose. This analogue does not carry the disavantage of being metabolized to products that could falsely be believed to be glucose after measurement of radioactivity in the venous effluent.

In the present study we have simultaneously measured the net glucose uptake and the transport rate of 2-deoxyglucose in the perfused rat hindlimb preparation under various conditions. For the whole range of glucose transport rates obtained, we found that the 2-deoxyglucose transport, i.e. penetration of the muscle membrane, was closely correlated with and always higher than the net uptake of glucose by approx. 20%. These results strongly suggest that the transport is not rate-limiting for net glucose uptake, but rather appears to be modulated in such a way that there is a fixed relationship between transport and uptake. The linkage between the transport and uptake process of glucose is a concept rather different from the classical one of an inward step that is rate-limiting in its own right.

From the findings that glucose accumulates intracellularly during certain conditions, such as contractile activity, it was concluded that, when muscle is stimulated

to contract, the site of regulation of the glucose uptake shifts from transport across the cell membrane to glucose phosphorylation (Kipnis & Cori, 1959; Berger *et al.*, 1975; Rennie & Holloszy, 1977). Cheung *et al.* (1978) have found the same kind of shift in insulin-stimulated hearts. In adipocytes and in adipose plasma-membrane vesicles, Foley & Hueckstead (1984) have demonstrated that glucose 6-phosphate decreased hexose transport rates and that this effect was not via competitive inhibition. They suggested an intracellular regulation of glucose transport by glucose metabolites. The commonly used technique for investigating glucose transport is based on determination of the distribution volume after equilibrating the tissue with glucose and an extracellular marker. This technique does not allow rapid and repeated measurements and is rather imprecise to the technical difficulty in determining a low intracellular glucose concentration accurately.

Our hypothesis is that the two processes are regulated together by the metabolic demands of the muscle cells. The support for this theory is that hypoxia and contractile activity stimulates the transport rate in an identical way, i.e. the same transport rates are achieved with either hypoxia or contractions at the same glucose concentration. In common for these two situations is that there are increased demands on the energy production, as reflected in altered metabolite concentrations, such as decreased PCr. The coupling between the metabolic state of the tissue and the rate of glucose transport is supported by the correlation between the intramuscular PCr concentration and the transport rate.

A correlation between the PCr concentration and transport rate was observed at 10 mM but not at 2 and 25 mM, i.e. only at a glucose concentration close to the physiological. The explanation for this is the high dependence of the glucose transport on the available glucose concentration under these circumstances. Thus, during hypoxia and contractile activity, the glucose transport rate (and the net glucose uptake) is a direct function of the glucose concentrations within the range 1–50 mM (Figs. 3 and 4). A high glucose concentration such as 25 mM will therefore stimulate the glucose transport rate itself, and the excess will be incorporated into glycogen, as demonstrated in Table 2. At a low glucose concentration, e.g. 2 mM, the exogenous glucose supply will limit the transport rate, and endogenous muscle glycogen will be utilized to a higher extent. At glucose concentrations far from the physiological, the correlation between the metabolic state of the tissue and the transport rate will therefore be obscured by the high dependence of the transport rate on the glucose concentration itself. These findings are in agreement with those in one of our previous studies, where significant relationships between the net glucose uptake and the intramuscular energy state, determined as the PCr concentration, the ATP/ADP ratio and the lactate/pyruvate ratio, were obtained in the perfused contracting rat hindlimb at a physiological glucose concentration (Walker *et al.*, 1982).

Under conditions when the transport rate reaches substrate saturation, the application of enzyme kinetics to the transport curves gives the possibility to calculate the K_m as well as the $V_{max.}$ of the transport process. We have previously shown that the 2-deoxyglucose transport is half-maximally inhibited (K_i) at a perfusate glucose concentration of about 10 mM in fed animals (Rennie *et al.*, 1983). This may be considered as numerically equal to the K_m of glucose transport and thus a measurement of the carrier affinity. The carrier affinity was previously shown to be decreased by starvation, but was unaffected by insulin. The calculated $V_{max.}$ provides a measurement of the maximal transport rate and thus the carrier availability. The carrier availability was previously shown to vary considerably with feeding and starvation, and also with insulin (Rennie *et al.*, 1983).

Owing to the possibility of a non-steady state in energy metabolism during long-term muscle stimulation, a protocol where the whole range of glucose concentrations was to be analysed during contractile activity was not considered possible. An alternative way to distinguish between an increased transport rate being elicited by an increase in carrier affinity and/or carrier availability is to compare 2-deoxyglucose transport under the following two conditions: at a perfusate glucose concentration at which the glucose transport is likely to be substrate-saturated (25 mM) and one well below that value (2 mM). If contractile activity, alone or together with hypoxia, stimulates sugar transport by an increase in carrier affinity, a major increase in glucose transport would be observed at 2 mM-glucose. If transport stimulation were predominantly due to an increased carrier availability, an enhanced transport would be observed at 25 mM-glucose. If the effect was due to an increase in both carrier affinity and carrier availability, then enhancement of transport would occur at both the high and the low glucose concentration.

The greater stimulation of 2-deoxyglucose transport induced by hypoxia and contractile activity at high rather than low perfusate glucose concentration strongly suggests that the major stimulatory effect of hypoxia and muscle contraction is exerted by an increase in carrier availability. However, these results do not rule out smaller changes in carrier affinity, since a full kinetic analysis over a larger range of glucose concentration could not be performed. Nevertheless a decrease in K_m, i.e. increase in carrier affinity, is unlikely. We have previously observed that the effect of insulin in this preparation is to increase the maximal transport rate without affecting the apparent carrier affinity. Our present results indicate that this is also the case for hypoxic and contractile stimulation of the transport process. Thus it appears likely that all three stimuli work through a common carrier mechanism. The lack of additive enhancement of sugar transport with maximal hypoxia or contractile stimuli, and the observation that stimulation by hypoxia or contractions does not require the presence of insulin, give further support to this conclusion.

It has been suggested that insulin has a permissive effect on the enhancement of glucose transport observed with anoxia and contractile activity in isolated incubated soleus muscles as well as perfused rat hindlimb muscle (Chaudry & Gould, 1969; Berger *et al.*, 1976). The hindlimb was therefore perfused in the absence and in the presence of physiological concentrations of insulin (0.1 unit/l) during hypoxia or contractile activity, and in disagreement with the above quoted results we found a stimulation of 2-deoxyglucose transport irrespective of the presence or absence of insulin. We were unable to detect endogenous insulin by radioimmunoassay (< 0.005 unit/l) in the venous perfusate in the absence of added insulin. Further, the transport rate achieved

when no insulin was added was one-fifth of that at 0.01 unit/l (Fig. 3). In a study on diabetic rats Wallberg-Henriksson & Holloszy (1984) also found that the stimulatory effect of muscle contraction on glucose uptake by perfused rat muscles did not require the presence of insulin.

In conclusion, the present results provide a new view of the metabolic regulation of glucose transport versus glucose uptake in skeletal muscle tissue. The two processes are closely matched, and the transport rate is never limiting for the net uptake. The stimulatory effects of insulin, hypoxia and contractile activity are all exerted through an increase in the carrier availability, which, together with the fact that these stimuli are not additive, suggests that they are mediated by a common mechanism. Furthermore, results are presented supporting regulation of the transport and uptake process by the metabolic demands of the muscle cells during hypoxia and contractile activity, a hypothesis that requires further investigation.

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REFERENCES

Atkins, G. L. & Gardner, M. L. G. (1977) Biochim. Biophys. Acta **468**, 127–145

Berger, M., Hagg, G. & Ruderman, N. B. (1975) Biochem. J. **146**, 231–238

Bylund-Fellenius, A.-C., Idström, J.-P., Rennie, M. J. & Scherstén, T. (1982) J. Physiol. (London) **336**, 18*P*–19*P*

Chaudry, I. H. & Gould, M. K. (1969) Biochim. Biophys. Acta **177**, 527–534

Cheung, J. Y., Connover, C., Regen, D. M., Whitfield, C. F. & Morgan, H. E. (1978) Am. J. Physiol. **234**, E70–E78

Foley, J. E. & Huecksteadt, T. P. (1984) Biochim. Biophys. Acta **805**, 313–316

Goodman, M. N. & Ruderman, N. B. (1979) Am. J. Physiol. **235**, E519–E523

Hultman, E. (1967) Scand. J. Clin. Lab. Invest. **19**, 209–217

Idström, J.-P., Harihara Subramanian, V., Chance, B., Scherstén, T. & Bylund-Fellenius, A.-C. (1985) Am. J. Physiol. **248**, H40–H48

Kipnis, D. M. & Cori, C. F. (1959) J. Biol. Chem. **234**, 171–176

Morgan, H. E. & Whitfield, C. F. (1974) Curr. Top. Membr. Transp. **4**, 255–272

Morgan, H. E., Henderson, M. J., Regen, D. M. & Park, C. R. (1961) J. Biol. Chem. **236**, 253–264

Randle, P. J. & Smith, G. H. (1958) Biochem. J. **70**, 490–500

Rennie, M. J. & Holloszy, J. O. (1977) Biochem. J. **168**, 161–170

Rennie, M. J., Idström, J.-P., Mann, G. E., Scherstén, T. & Bylund-Fellenius, A.-C. (1983) Biochem. J. **214**, 737–743

Richter, E. A., Garetto, L. P. & Goodman, M. N. (1982) J. Clin. Invest. **69**, 785–793

Szabo, A. J., Mahler, R. J. & Szabo, O. (1969) Horm. Metab. Res. **7**, 156–161

Walker, P. M., Idström, J.-P., Scherstén, T. & Bylund-Fellenius, A. C. (1982) Eur. J. Appl. Physiol. **48**, 163–176

Wallberg-Henriksson, H. & Holloszy, J. O. (1984) J. Appl. Physiol. **57**, 1045–1049

Yudilevich, D. L. & Mann, G. E. (1982) Fed. Proc. Fed. Am. Soc. Exp. Biol. **41**, 3045–3053