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Effects of insulin on glucose transport and glucose transporters in rat heart

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The effect of insulin on glucose transport and glucose transporters was studied in perfused rat heart. Glucose transport was measured by the efflux of labelled 3-*O*-methylglucose from hearts preloaded with this hexose. Insulin stimulated 3-*O*-methylglucose transport by: (*a*) doubling the maximal velocity ($V_{max.}$); (*b*) decreasing the K_d from 6.9 to 2.7 mM; (*c*) increasing the Hill coefficient toward 3-*O*-methylglucose from 1.9 to 3.1; (*d*) increasing the efficiency of the transport process (*k* constant). Glucose transporters in enriched plasma and microsomal membranes from heart were quantified by the [^{3}H]cytochalasin-B-binding assay. When added to normal hearts, insulin produced the following changes in the glucose transporters: (*a*) it increased the translocation of transporters from an intracellular pool to the plasma membranes; (*b*) it increased (from 1.6 to 2.7) the Hill coefficient of the transporters translocated into the plasma membranes toward cytochalasin B, suggesting the existence of a positive co-operativity among the transporters appearing in these membranes; (*c*) it increased the affinity of the transporters (and hence, possibly, of glucose) for cytochalasin B. The data provide evidence that the stimulatory effect of insulin on glucose transport may be due not to the sole translocation of intracellular glucose transporters to the plasma membrane, but to changes in the functional properties thereof.

INTRODUCTION

The muscle mass represents the main target of insulin on glucose disposal in the whole body. Contradictory data have been reported concerning the effect of this hormone on these tissues. Thus the effect of insulin on glucose transport in various types of muscles (including the heart) has been attributed to an increase in the maximal velocity ($V_{max.}$) of the transport, without any change in its affinity for glucose [1,2], or to an increase in $V_{max.}$ of transport while the affinity for glucose is decreased [3–5]. It has also been reported that insulin did not cause any change in the $V_{max.}$ of transport, while increasing (2–3-fold) the affinity of the process [6,7] for its substrate.

In adipose cells [8,9] and in isolated diaphragm [10], one mechanism underlying the insulin-induced increase in the $V_{max.}$ of glucose transport has been shown to be an increase in the translocation of glucose-transporter molecules from an intracellular pool to the plasma membrane. However, the number of glucose transporters translocated to the plasma membrane under the influence of insulin may not completely explain the increase in the $V_{max.}$ of D-glucose transport in itself [8,9,11]. The discrepancy between the values of actual transport of D-glucose in intact cells and those of the number of glucose transporters measured in the plasma membrane is illustrated by the observations that insulin stimulates the former process by 5–35-fold [12–16], but the latter by 2–4-fold only [8,9,11]. One hypothesis could be that the translocation process may be only a part of the overall mechanisms by which insulin stimulates glucose uptake.

The aim of the present study was to reconsider the way by which insulin stimulates D-glucose transport in one type of muscle, i.e. the heart. To do so, the actual D-glucose transport kinetics were first defined in the perfused rat organ, by using the technique of 3-*O*-methyl-D-glucose efflux [17], in the absence and in the presence of insulin. Subsequently, the data obtained were compared with the changes in the characteristics of the glucose-transporter system observed in enriched plasma and microsomal membranes.

EXPERIMENTAL

Animals

Normal male rats (15 weeks old) of the Zucker strain (*FA/FA*) bred in our laboratory were used. They had free access to a standard laboratory chow (UAR, Epinay/Orge, France) and were maintained at a constant temperature (23 °C) in animal quarters with a 12 h artificial light cycle.

Heart perfusion and 3-*O*-methylglucose-transport studies

Rats were anaesthetized with pentobarbital (90 mg/kg). The heart was rapidly removed, placed in ice-cold 0.9% NaCl solution for 10 s, cannulated via the aorta and perfused by the Langendorff method [17–19]. The perfusion medium consisted of a Krebs–Ringer bicarbonate buffer (118 mM-NaCl, 4.8 mM-KCl, 1.2 mM-KH_2PO_4, 1.2 mM-$MgSO_4$, 2.5 mM-$CaCl_2$, 24.9 mM-$NaHCO_3$) at pH 7.4. When 3-*O*-methylglucose transport was investigated, glucose was replaced by pyruvate (5 mM) as energy source [20]. To measure glucose transport itself, hearts were pre-perfused and perfused with or without insulin (10 min) with a recirculating medium containing 6 mM-3-*O*-methyl-D-[^{14}C]glucose (0.3 μCi/ml), and 6 mM-L-[^{3}H]glucose (0.3 μCi/ml) (loading

period). Labelled L-glucose was used to calculate the extracellular and free diffusion components. 3-*O*-Methylglucose was in the intra- and the extra-cellular space, and L-glucose mainly in extracellular space and to some extent (via simple diffusion) in the intracellular space. 3-*O*-Methylglucose counter-transporter via facilitated diffusion (efflux) could be calculated by subtracting, for each vial, total labelled L-glucose content from total labelled 3-*O*-methylglucose content.

When the effect of 10 munits of insulin/ml was studied, the hormone was added to both pre-perfusion and perfusion media. This high hormone concentration represents the maximal stimulating dose of insulin in heart [17]. When basal 3-*O*-methyl-D-glucose efflux rates were investigated (without added insulin) a pre-perfusion period of 10 min was allowed to wash away endogenous insulin [17].

All hearts were perfused at low pressure (50 mmHg), a situation in which the effect of insulin on glucose uptake is maximal [17].

Heart plasma and microsomal membrane preparation

For membrane preparation, hearts were perfused for 15 min in the presence or in the absence of insulin and at 6 mM-glucose concentration in the medium. Hearts were then disconnected from the perfusion apparatus and immediately frozen in liquid N_2. At the time of membrane preparation, hearts were transferred into liquid N_2 and powdered in a cooled mortar. The powder obtained was homogenized in 4 ml of buffer A (10 mM-$NaHCO_3$/5 mM-NaN_3, pH 7.0) with a glass pestle (B. Braun, Molsungen, Germany) connected to a homogenizer (Heidolph model R2R1). Samples (50 μl) of homogenate were used for protein and marker-enzyme measurements. The homogenate was centrifuged for 20 min at 7000 ***g*** in a Sorvall RC2-B centrifuge. The resulting pellet (P1) was kept for the preparation of plasma membrane, whereas the supernatant (S1) permitted us to obtain purified microsomal membranes.

The first pellet, P1, was resuspended with a syringe in 2 ml of buffer B (10 mM-Tris/HCl, pH 7.4) and centrifuged for 20 min at 1000 ***g***. The supernatant was spun for 20 min at 48000 ***g***. The pellet thus obtained was resuspended in 1 ml of medium B and placed on 20% (v/v) Percoll (Pharmacia, Uppsala, Sweden) solution in buffer C (255 mM-sucrose/10 mM-Tris/HCl/2 mM-EGTA), centrifuged for 1 h in an ultracentrifuge (Beckman L5-50) at 55000 ***g*** in a SW27 rotor. An opaque band corresponding to a density of 1.030 g/ml, as measured with density-marker beads (Pharmacia), was removed. The plasma membranes thus obtained were washed in medium B (see above) and centrifuged for 1 h in an ultracentrifuge at 170000 ***g***, in a 50 Ti rotor (giving pelleted plasma membranes).

The supernatant S1 was centrifuged for 20 min at 48000 ***g***. The resulting supernatant was placed on top of 20% Percoll in buffer C and ultracentrifuged for 1 h at 55000 ***g*** in a SW27 rotor. Here, a band, corresponding to a density of 1.036 g/ml, as measured with density-marker beads, was collected. The microsomal membranes thus obtained were washed with medium B (as above), and ultracentrifuged for 1 h at 170000 ***g*** in a 50 Ti rotor (giving pelleted microsomal membranes).

The pellets of both types of membranes were resuspended in 1 ml of buffer C, of which 0.7 ml was kept for cytochalasin-B-binding assays. The remaining 0.3 ml was saved for the measurement of marker enzymes and total proteins.

Marker enzyme assays

Total proteins were measured by the Bio-Rad method, with γ-globulin as standard [21]. 5′-Nucleotidase was the marker used for plasma membranes and was determined as described elsewhere [22]. NADPH–cytochrome *c* reductase, a marker of microsomal membranes, was determined as described in [23].

Cytochalasin-B-binding assays

Cytochalasin B binding was carried out on plasma and microsomal membranes obtained as described above, by using initially ten, and then five, concentrations of the ligand, i.e. 40, 80, 120, 160, 200, 240, 280, 320, 400, 500 nM, then 40, 80, 160, 320, 420 nM, partly by a previously validated technique [24,25]. When mentioned, the measured specific cytochalasin-B-binding activities were expressed as pmol/mg of protein and were adjusted to those that would have been observed, had the membrane fractions been free of cross-contamination. Such adjustments were based on specific activities of the enzyme markers and protein recoveries, with the assumption that 5′-nucleotidase activity is localized specifically in plasma membranes and that NADPH–cytochrome *c* reductase activity is specific for microsomal membrane. The total amount of glucose transporters was calculated on the basis of the raw data and taking into account the recovery of specific marker enzymes.

Glucose transporters were initially measured in plasma membranes from heart perfused with or without insulin, and the D-glucose-displaceable [^{3}H]cytochalasin B binding used at ten different concentrations (40–500 mM) was investigated. Subsequently, five cytochalasin B concentrations were used, as no significant differences in R_0 (number of transporters), Hill coefficient and K_d values could be detected whether the binding curves were carried out with ten or five cytochalasin B concentrations. Furthermore, the D-glucose-inhibitable [^{3}H]cytochalasin B binding was carried out on both plasma and microsomal membranes.

Statistics and calculations

The Hill equation [26] was used to analyse the data as described elsewhere [24,25]. All data were expressed as means ± S.E.M. for at least three independent experiments. Difference analyses were performed with the one-tailed Student's *t* test for unpaired data. The *P* values obtained indicate the probability that the two events are similar.

RESULTS

The data on 3-*O*-methyl-D-glucose transport and cytochalasin B binding were obtained with the same preparation (i.e. the perfused heart), and allowed the correlation of the kinetic characteristics for the actual transport of the hexose with the parameters of cytochalasin B binding.

Transport of 3-*O*-methyl-D-glucose

3-*O*-Methylglucose transport was measured at various concentrations of the hexose (1–15 mM), with or without insulin used at a maximal concentration (10 munits/ml). The results obtained are shown in Fig. 1. The maximal velocity of the transport ($V_{max.}$) was increased by insulin

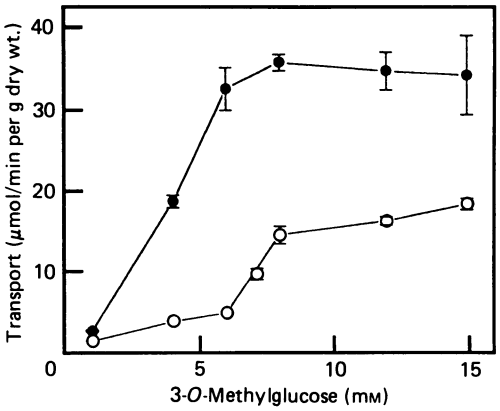

Fig. 1. Effect of insulin on initial 3-*O*-methylglucose efflux rates in rat hearts perfused with increasing concentrations of the hexose

○, Basal transport; ●, insulin-stimulated transport. Each point is the mean ± S.E.M. for three to six independent experiments. All insulin-stimulated data are statistically different ($P < 0.05$) from basal values (no insulin), except for the 1 mM-3-*O*-methylglucose point. Small S.E.M. values are contained within the symbols. Insulin concentration throughout was 10 munits/ml.

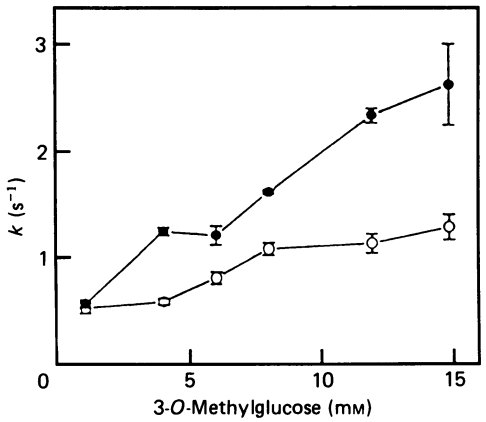

Fig. 2. Effect of insulin on the kinetic constant *k* (i.e. the fraction of hexose transported per unit of time) of 3-*O*-methylglucose in rat hearts perfused with increasing concentrations of the hexose

The *k* values were measured for each 3-*O*-methylglucose concentration, in the presence (●) or in the absence of insulin (○). Each point is the mean ± S.E.M. for three to six independent experiments. Small S.E.M. values are contained within the symbols. Insulin concentration was 10 munits/ml. All insulin-stimulated *k* values are statistically different from basal ones (no insulin), with *P* at least < 0.025, except for the 1 mM-3-*O*-methylglucose point (not significant).

from 17.6±0.7 (no insulin) to 34.1±1.2 μmol/min per g dry wt. ($P < 0.001$). Moreover, the half-maximal value (K_m) was decreased by insulin from 6.9±0.3 (controls) to 2.7±0.4 mM ($P < 0.001$). This change in K_m was moderate, but likely to be of importance, as it was observed at physiological glucose concentrations (5–7 mM). As further shown by Fig. 1, the dose/response curves were curvilinear, i.e. they differed from a rectangular hyperbola. For this reason, the curves were analysed in accordance with Hill [26]. A Hill coefficient of 1.93 ($r^2 = 0.88$) was obtained for the dose/response curve performed in the absence of insulin, whereas the coefficient was 3.08 ($r^2 = 0.93$) for the dose/response curve carried out in the presence of insulin ($P < 0.025$).

As shown in Fig. 2, the kinetic constant *k* (which represents the fraction of the hexose transported per unit of time, an index of the transport efficiency) was 0.5 in the absence or presence of insulin when 3-*O*-methylglucose was used at 1 mM concentration. For higher (4–15 mM) concentrations, *k* values increased in the absence of insulin (1.2±0.1), and the presence of the hormone further augmented all respective *k* values (2.6±0.3) ($P < 0.005$).

Glucose transporters as measured by the [^{3}H]cytochalasin-B-binding assay

To determine the role of glucose transporters in the regulation of D-glucose transport, [^{3}H]cytochalasin-B-binding assays were performed in plasma and microsomal

Table 1. Enzyme markers for enriched plasma and microsomal membranes obtained from control and insulin-stimulated perfused hearts of normal rats

Purified plasma and microsomal membranes were prepared as described in the Experimental section. Results are means ± S.E.M. for duplicate determinations in three independent experiments. Differences between basal and insulin-stimulated state were not significant.

	5′-Nucleotidase		NADPH–cytochrome *c* reductase		Protein	
	Sp. activity (μunits/mg)	Recovery (%)	Sp. activity (munits/mg)	Recovery (%)	(mg/g of heart)	Recovery (%)
Control						
Homogenate	1.7±0.1		3.2±0.5		158.6±10.4	
Plasma membranes	31.8±5.4	19.7±1.4	12.0±1.2	10.7±0.9	5.7±1.0	2.90±0.35
Microsomal membranes	2.4±1.0	4.6±0.2	21.1±3.1	25.2±1.3	2.4±0.2	1.29±0.16
Insulin (10 munits/ml)						
Homogenate	1.7±0.2		3.5±0.2		162.9±13.4	
Plasma membranes	29.1±4.5	20.0±2.0	11.6±0.9	11.3±0.9	5.5±0.8	2.61±0.21
Microsomal membranes	3.1±0.4	4.3±0.3	25.2±1.9	24.9±1.5	2.4±0.3	1.19±0.14

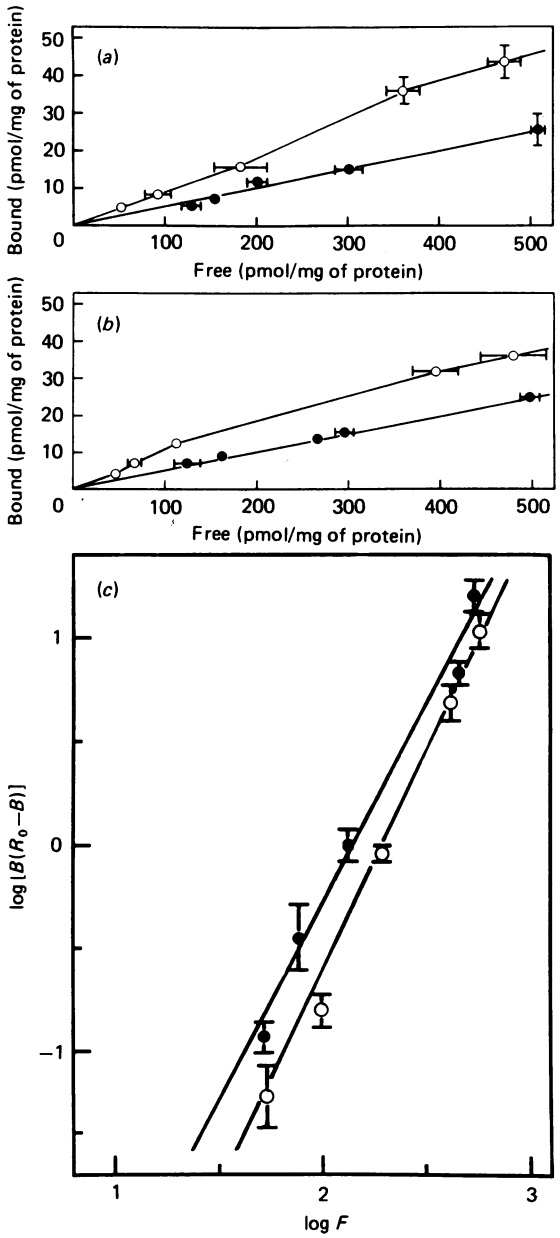

Fig. 3. [^{3}H]Cytochalasin-B-binding curves to microsomal membranes isolated from perfused rat hearts

Before preparation of membrane fraction, hearts were perfused for 15 min with 6 mM-D-glucose in the absence (*a*) or in the presence of 10 munits of insulin/ml (*b*). In each case, cytochalasin B binding was measured at five free cytochalasin B concentrations in the absence (○) or the presence (●) of 500 mM-D-glucose as described in the Experimental section. Results are plotted as bound (*B*) versus free (*F*) cytochalasin B (*a*, *b*). Each point is the mean ± S.E.M. for three to four independent determinations made in duplicate. The Hill plot (*c*) was constructed by subtracting each curve obtained in the presence of D-glucose from its respective curve obtained in the absence of D-glucose, and analysing the resulting values by linear regression: ●, +insulin; ○, no insulin.

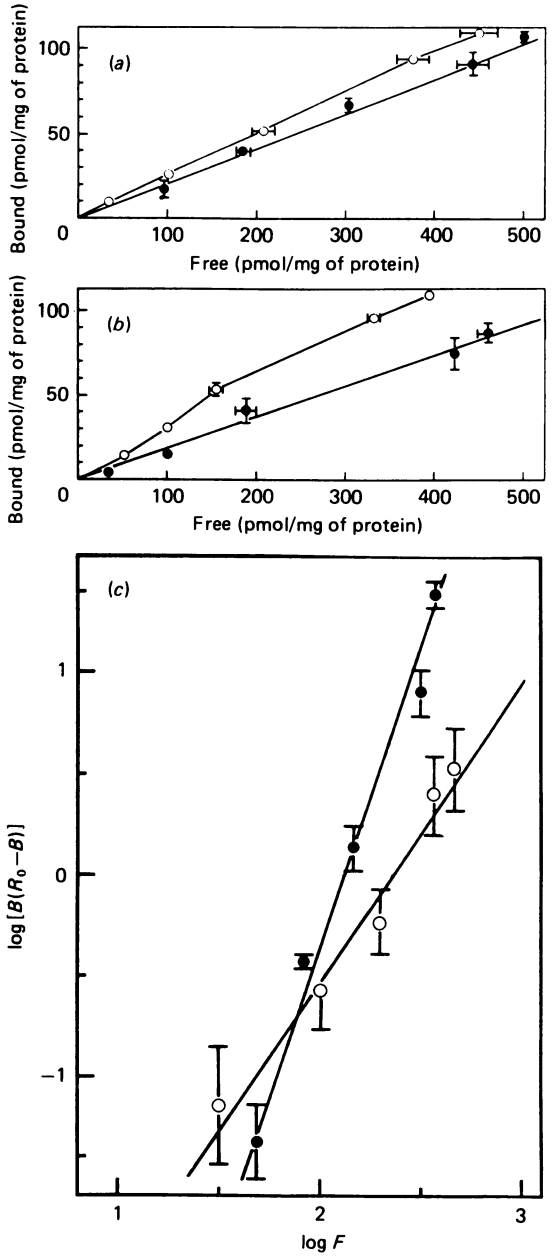

Fig. 4. [^{3}H]Cytochalasin-B-binding curves to plasma membranes isolated from perfused rat hearts

Before preparation of membrane fraction, hearts were perfused for 15 min with 6 mM-D-glucose in the absence (*a*) or in the presence of 10 munits of insulin/ml (*b*). In each case, cytochalasin B binding was measured at five cytochalasin B concentrations in the absence (○) or the presence (●) of 500 mM-D-glucose as described in the Experimental section. Results are plotted as bound (*B*) versus free (*F*) cytochalasin B (*a*, *b*). Each point is the mean ± S.E.M. for three to four independent determinations made in duplicate. The Hill plot (*c*) was constructed by subtracting each curve obtained in the presence of D-glucose from its respective curve obtained in the absence of D-glucose, and analysing the resulting values by linear regression: ●, +insulin; ○, no insulin.

membranes obtained from hearts perfused with or without insulin. The enzyme markers of the respective membranes (plasma and microsomal) were determined to evaluate both the quantity and the purity of each membrane fraction. As shown by Table 1, 5′-nucleotidase was used as plasma-membrane marker and NADPH–cytochrome *c* reductase as microsomal-membrane marker. The results indicated that microsomal mem-

Table 2. Effects of insulin on number of glucose transporters, Hill coefficient and dissociation constant (K_d) in plasma and microsomal membranes obtained from control and insulin-stimulated perfused hearts of rats

Total and corrected number of transporters were measured by calculation from [^{3}H]cytochalasin B binding are described in the Experimental section. Results are means ± S.E.M. for three to four independent experiments made in duplicate. * indicates insulin-stimulated value different from basal, with at least $P < 0.05$.

	No. of glucose transporters				
	Raw data (pmol/mg of protein)	Corrected for contamination (pmol/mg of protein)	Total binding (pmol/g of tissues)	Hill coefficient	K_d (nM)
Plasma membranes					
Basal	17.8±4.1*	10.0±2.5*	541±125*	1.6±0.2*	230±26*
Insulin	37.0±3.2	40.5±4.3	1027±89	2.7±0.1	136±9
Microsomal membranes					
Basal	22.5±4.0*	22.4±3.8*	298±33*	2.2±0.2	187±16*
Insulin	13.7±0.8	13.3±0.7	131±8	1.9±0.1	165±7
Total					
Basal	40.4±5.7		838±110*		
Insulin	50.7±3.0		1159±86		

branes were purified with a contamination of 7% by plasma membranes. Plasma membranes were contaminated by microsomal ones to a larger extent, i.e. by 46%. Neither the amount of each membrane marker, nor its purification, was changed by insulin (results not shown).

The curves of cytochalasin B binding measured in microsomal fractions from hearts perfused in the absence or in the presence of insulin are shown in Fig. 3, which represents both the total and the non-specific binding (upper panel). Specific binding was obtained by the difference between total and non-specific binding and was analysed in accordance with Hill. By doing so, a maximal binding of 22.5 pmol/mg of protein was obtained in the absence of insulin, which decreased to 13.7 pmol/mg of protein in its presence, as shown in Table 2. With insulin, the Hill coefficient of microsomal membranes was not significantly changed (2.2 in basal state and 1.9 with insulin). The K_d (i.e. the mean affinity of cytochalasin B for the glucose transporters) measured in microsomal fractions also remained unchanged, i.e. 187 nM in the absence and 165 nM in the presence of insulin. The correction for cross-contamination did not change the value for glucose-transporter number, as microsomal membranes were contaminated by only 7% with plasma membranes (Tables 1 and 2).

The data on [^{3}H]cytochalasin B binding obtained in plasma membranes from hearts perfused with or without insulin are depicted in Fig. 4. Here again the two upper panels represent total and D-glucose-displaceable binding, with or without the hormone. Specific binding obtained gave the following raw data: a maximal binding of 17.8 pmol/mg of protein in the basal state, which was increased to 37.0 pmol/mg of protein by insulin. Insulin also increased the Hill coefficient from 1.6 to 2.7, whereas K_d values decreased in the presence of the hormone from 230 to 136 nM (Table 2). Finally, from the number of glucose transporters present in the microsomal and the plasma membranes of perfused hearts with or without insulin, the total number and the respective changes observed in the presence of the hormone could be measured, as shown by Table 2. The total amount of glucose transporters (i.e. those of plasma membranes plus those of microsomal membranes) was 838 pmol/g of heart in basal conditions and 1159 pmol/g of heart in the presence of insulin. Moreover, insulin decreased the glucose transporter number in microsomes (from 298 to 131 pmol/g) and increased those of the plasma membrane (from 541 to 1027 pmol/g). Note that the correction for cross-contamination mainly affected plasma-membrane transporters, as they were contaminated by 46% by microsomal membranes.

DISCUSSION

3-*O*-Methylglucose transport

The present data clearly show that, in perfused heart from normal rats, insulin stimulates 3-*O*-methylglucose transport by increasing the maximal velocity ($V_{max.}$), as well as by decreasing the K_m of the glucose transport. When tested as a function of 3-*O*-methylglucose concentrations, the resulting uptake curves of the hexose were sigmoidal. For this reason, the data were analysed by the Hill equation. Such analysis indicates the occurrence of a positive co-operativity within the glucose transporters, in the presence of insulin (Hill coefficient increased from 1.9 to 3.1). This is in keeping with the existence of an increased k value in the presence of insulin (Fig. 2), reflecting an increased efficiency of the transporting system [27].

The present study also shows that, under basal conditions, 3-*O*-methyl-D-glucose has an effect on the regulation of its own transport, the dose–effect relationship being sigmoidal, as mentioned above (Fig. 1), in contrast with what has been observed in isolated cardiocytes, where the reported velocity curves were hyperbolic [28]. The observation of a Hill coefficient of 1.9 in the basal state (no insulin) suggests the presence either of a dimeric glucose transporter, as shown by others [29], or of a co-operativity between these two sites. This finding is also in keeping with the observation that the kinetic constant k is increased by increasing 3-*O*-

methylglucose concentrations, although such an increase is less marked than that observed in the presence of insulin (Fig. 2).

Glucose transporters as studied by cytochalasin B binding

In the literature, almost all the studies of D-glucose-inhibitable binding of labelled cytochalasin B carried out to evaluate the function of glucose transporters have yielded linear Scatchard plots [8,10,30]. No change in K_d was observed whether insulin was present or not. Those studies demonstrated that the effect of insulin on glucose transport was due to the translocation of glucose transporters from some intracellular pool to the plasma membrane, a reversible process that did not alter the properties of the transporters.

In the present work, binding of cytochalasin B to heart plasma and microsomal membranes resulted in downward-concave Scatchard plots. For this reason, their analysis was carried out in accordance with Hill, thereby yielding three main functional values of glucose transporters, i.e. R_o (= number of transporters), Hill coefficient (presence or absence of co-operativity) and K_d (changes in the affinity for cytochalasin) for the two respective types of membranes. By doing so, and in agreement with others [8,10,30,31], it was observed that insulin doubled the number of glucose transporters present in the plasma membrane, an effect that was accompanied by a concomitant decrease in the number of glucose transporters of the microsomal membranes. Insulin increased the total number of glucose transporters (per heart) by 25%, an effect that was unlikely to be related to synthesis *de novo*, as the exposure to insulin was only 15 min, and is attributed to recruitment of masked transporters. As the 3-*O*-methylglucose transport in normal hearts was stimulated 8-fold in the presence of insulin when tested under the same experimental conditions (i.e. 50 mmHg pressure and 6 mM-hexose), factors other than the mere translocation had to participate in such a stimulation of the transport. The Hill analysis provided information for at least two additional parameters (Table 2). The Hill coefficient of the plasma-membrane glucose transporters was increased by insulin, from 1.6 to 2.7. This indicated the occurrence of a co-operativity between different sites of the D-glucose transporters, an observation that is in keeping with the suggestion that the glucose transporters may exist in mono-, di- and tetra-mer configurations [29]. In microsomal membranes, the Hill coefficient remained unchanged by the addition of insulin. This suggests a dimeric transporter structure [29], or two classes of transporter, as described previously [32,33]. It is possible that one class of transporter would be translocated to the plasma membrane and then be modified therein for further activation [32,33]. Finally, insulin did not change K_d values of microsomal membranes, but the value was clearly decreased by insulin once the transporters were in the plasma membranes (from 230 to 136 nM).

The present study therefore suggests that insulin stimulated the translocation of transporters from an intracellular pool to the plasma membrane and also modified the properties of the transporters translocated to the plasma membrane (increased Hill coefficient, increased affinity for cytochalasin B), possibly via effects that could be related to changes in glycosylation [32,33] of the glucose transporters that would render them more efficient. Another study on adipocytes also suggested a dissociation between insulin stimulation of glucose transport activity and the recruitment of glucose transporters by the hormone [34].

Putting together the effects of insulin on actual 3-*O*-methyl-D-glucose transport and those pertaining to the glucose transporters, it may be concluded that the translocation process is responsible for only part of the increased glucose transport. The analogous augmentation, in insulin-treated hearts, of Hill coefficients observed for both the 3-*O*-methylglucose transport and the plasma-membrane glucose transporters, the increased affinity of the transport process for 3-*O*-methylglucose together with that of the cytochalasin B in the plasma membrane (possibly reflecting an increased affinity of their particular transporters for glucose) suggest that, once translocated in the plasma membrane by insulin, the transporters change their intrinsic activity and become more efficient. This is reflected by the insulin-induced augmentation of the kinetic constant k, indicating that, in the presence of the hormone, the fraction of D-glucose transported per unit time becomes much higher. Thus these data give new insights into the mechanisms responsible for the apparent discrepancy between actual glucose transport, which is stimulated to a greater extent by insulin than is the translocation of the transporters to the plasma membrane. They suggest that this discrepancy can be attributed to insulin-induced changes in the properties of the glucose transporters once present in the plasma membrane, changes which have been so far largely overlooked.

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