Insulin action on glucose transport in isolated cardiac myocytes: signalling pathways and diabetes-induced alterations

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Stimulation of glucose transport by insulin has been recognized to represent one of the most prominent actions of this hormone in muscle and adipose tissue [1]. The molecular mechanisms of insulin signal transduction have been the focus of extensive investigations during recent years (for a review, see [2]). Available data suggest a complex chain of events involving tyrosine kinase activity of the insulin receptor and possibly additional elements like G-proteins, protein kinase C and mediator molecules.

In contrast to adipose tissue, the molecular basis of insulin action on cardiac glucose transport remains poorly understood owing to the complexity of the available model systems. This laboratory has approached these problems by using freshly isolated and primary cultured adult rat cardiac myocytes, resulting in a detailed description of cardiac insulin receptors [3-5] and insulin action on the glucose transporter [6-8]. In the present paper, we report on the mechanisms of insulin transmembrane signalling and diabetes-induced alterations.

Early events in cardiac insulin action

Insulin receptor tyrosine kinase activity has now been identified as an integral part of the signal transduction pathway [9]. Autophosphorylation of cardiac insulin receptors was studied on wheat germ agglutinin–agarose-purified receptor preparations. Phosphoproteins were separated by SDS/PAGE, analysed by autoradiography and identified by laser-scanning densitometry. Insulin maximally stimulated phosphorylation of the β-subunit (M, 87 000) 4-fold with a half-maximal action at 3 × 10^-10 mol/l (Fig. 1). This agrees well with the dose–response relationship for activation of glucose transport by insulin in this cellular system [7]. Insulin was found to decrease the Kₘ of the receptor kinase for ATP from 34 to 9 μM, suggesting conformational changes upon binding of the hormone.

Autophosphorylation may be followed by a complex chain of events involving receptor-mediated endocytosis, lysosomal degradation and recycling of internalized receptors.

Fig. 1. Dose-response relationship for insulin-stimulated autophosphorylation of insulin receptors purified from isolated cardiac myocytes

The receptor material was incubated for 30 min with the indicated insulin concentrations followed by SDS/PAGE and laser-scanning densitometry of the phosphoprotein bands. Data are mean values ± S.E.M. of two to five separate receptor preparations.

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[8]. To rule out a functional relationship between insulin processing and stimulation of cardiac glucose transport, the kinetics of insulin internalization have been determined and correlated to the onset of insulin action. Internalization of the hormone increased linearly up to 10 min followed by a slower phase up to 60 min. A similar time course has been observed for the onset of insulin action with a rapid phase up to 8 min followed by a much slower phase, reaching maximal transport stimulation by 30–60 min. This striking kinetic similarity points to a possible functional association of the two reactions. This assumption has been substantiated by selectively blocking internalization of the hormone. Thus, inhibition of internalization by treatment of cardiocytes with phenylarsine oxide totally abolished initial insulin action on glucose transport which remained unaffected by the lysosomal inhibitor chloroquine. The latter drug, however, was found to reduce steady-state insulin action by 60%. From these data, it may be concluded that very early processing of insulin receptor complexes and subsequent degradative steps may be involved in mediating the stimulatory action of insulin on the glucose transport system of ventricular cardiomyocytes.

Studies on the role of protein kinase C

It has been suggested that protein kinase C is involved in the stimulation of glucose transport by insulin (for a review, see [10]). This assumption is supported by an enhancement of glucose transport by phorbol esters, which has been observed in a variety of cell types. It remains controversial, however, if transport stimulation by insulin and phorbol esters is mediated by common pathways. Additional evidence for the involvement of protein kinase C in the mechanism of insulin action stems from observations on increased diacylglycerol production by insulin [11].

In the present study, the diacylglycerol content in cardiac myocytes was determined after conversion to phosphatidic acid by diacylglycerol kinase. As shown in Fig. 2, incubation of cells with insulin for 30 min resulted in a significant (P<0.05) increase in diacylglycerol content, whereas phospholipase C produced a considerable increase under the same conditions. The possible involvement of protein kinase C in the stimulatory action of insulin was further elucidated

![Graph 1](Figure 2) Effect of insulin on diacylglycerol content of isolated cardiac myocytes

Cells were incubated for 30 min with insulin (3.5 x 10^{-7} mol/l) or phospholipase C (0.2 units/ml). Diacylglycerol was then determined radioenzymatically in lipid extracts by conversion to phosphatidic acid using diacylglycerol kinase. Data are means ± S.E.M. (n = 3). * Significantly different from control (P<0.05).

![Graph 2](Figure 3) Effects of diabetes

Diabetes mellitus is known to be associated with a large variety of biochemical abnormalities of the myocardium. However, it is not clear if alterations in transmembrane signalling may be responsible for the pathogenesis of cardiac insulin resistance. Cardiac myocytes from streptozotocin diabetic rats and from genetically obese Zucker rats have been used to gain insights into such a relationship.

Stimulation of glucose transport was reduced by 64% in cardiac myocytes from streptozotocin diabetic rats with a concomitant decrease in basal transport by 29% (Fig. 3). The sensitivity of insulin-stimulated glucose transport to cholera toxin was completely lost in this insulin-deficient state. This may be due to alterations in the G-protein coupling the insulin receptor to the glucose transporter or to changes in the transporter itself. The latter possibility was studied by measuring mRNA expression of the insulin-responsive glucose transporter (IRGT). This was achieved by Northern blot analysis of total RNA isolated from the cardiocytes using a 24-base-pair oligonucleotide probe

![Graph 3](Figure 3) Effects of streptozotocin-induced diabetes on basal and insulin-stimulated 3-O-methylglucose transport and on amounts of glucose transporter mRNA

mRNA was quantified from autoradiograms by laser-scanning densitometry relative to the amounts in controls, which was assigned a value of 1.0. Data are means ± S.E.M. of at least four separate experiments. C, control; D, diabetes.
Aspects of the regulation of glucose transport in insulin-sensitive tissues in normal conditions and in type-2 diabetes

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Normal conditions

It has been shown previously that insulin stimulates glucose uptake in isolated adipocytes by favouring the translocation of glucose transporters from an intracellular pool (microsomes) to the plasma membrane [1]. The same process has been subsequently shown to occur in the muscle (diaphragm) [2]. More recently, it was demonstrated by using brown adipose tissue from normal and glucose transporter-deficient rats [3, 4] that, although the translocation process was a fundamental property of the glucose transport system, it did not account for all the changes observed. In particular, both in brown adipose tissue [4] and in the heart [5], insulin activated the transporters once translocated into the plasma membrane. This activation was evidenced by insulin-induced increases in the affinity of the plasma membrane transporters for cytochalasin and in the Hill coefficient thereof [6], indicative of the possible presence of a positive co-operativity among transporters [3–5].

In muscles, the study of glucose transport and, to a greater extent, that of the glucose transporter system, has been hampered until recently by technical difficulties that have now, at least to a large extent, been overcome [6, 7]. However, the preparation of purified muscle plasma and microsomal membranes in amounts sufficient to measure glucose transporters by labelled cytochalasin B binding is still complex [6, 7]. This is the reason why, although the heart is barely representative of muscle mass, it has been chosen to measure both glucose transport and glucose transporters.

It has been shown that muscle uptake (measured via its analogue 3-O-methyl-D-glucose) was stimulated by insulin and, in the absence of the hormone, by increasing the work-load of the organ [5, 8].

The g-glucose-inhibitable cytochalasin-binding assay (a method enabling the measurement of the amount of glucose transporters and the properties thereof) was then performed on enriched plasma and microsomal membranes obtained from perfused hearts [8]. The cytochalasin B-binding assay has been in particular, applied to plasma membranes obtained from hearts perfused with or without insulin. The amount of glucose transporters measured in the basal state was 24.6 pmol/mg protein and 41.2 pmol/mg protein when insulin (used at maximal concentrations) was present. Insulin also favoured, as detailed elsewhere [5], the translocation of heart glucose transporter from microsomal to plasma membranes. Furthermore, insulin changed the properties of the glucose transporters once present in the plasma membrane: the dissociation constant of cytochalasin B binding decreased from 208 nmol/l under basal conditions to 98 nmol/l in the presence of insulin, indicating a putative increase in the affinity of glucose transporters for cytochalasin B. The Hill coefficient, an index of co-operativity between transporters, was augmented in plasma membranes from a basal value of 1.5 (control hearts) to 2.2 (insulin-treated heart). The increase in workload (from 50 to 100 mmHg perfusion pressure) had an insulin-like effect upon the redistribution of the glucose transporters between the microsomal and the plasma membranes: it favoured the appearance of transporters in the plasma membranes, while decreasing those present in microsomal membranes.

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