G-protein-mediated regulation of the insulin-responsive glucose transporter in isolated cardiac myocytes

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Isolated muscle cells from adult rat heart were used to study the involvement of G-proteins in the regulation of the glucose transporter by insulin and isoprenaline. Efficient modification of G-protein functions was established by measuring isoprenaline-stimulated cyclic AMP production, viability and ATP content after treating the cells with cholera toxin and pertussis toxin for 2 h. Under these conditions cholera toxin decreased the stimulatory action of insulin on 3-O-methylglucose transport by 56%, but pertussis toxin had no effect. Basal transport was not affected by toxin treatment. Isoprenaline increased 3-O-methylglucose transport by 63%. This effect was not mimicked by dibutyryl cyclic AMP, but was completely blocked by cholera toxin. Streptozotocin-diabetes abolished isoprenaline action and decreased stimulation of transport by 64%. Concomitantly, cholera-toxin sensitivity of glucose transport was lost in cells from diabetic animals. This was paralleled by a large decrease (87 ± 4%) in mRNA expression of the insulin-regulatable glucose transporter, as shown by Northern-blot analysis of RNA isolated from cardiomyocytes of diabetic rats. These data suggest a functional association between the insulin-responsive glucose transporter and a cholera-toxin-sensitive G-protein mediating stimulation by insulin and isoprenaline.

INTRODUCTION

Insulin receptors are known to possess an intrinsic protein tyrosine kinase activity which, upon binding of the hormone, catalyses autophosphorylation of the β-subunit as well as phosphorylation of a variety of cellular proteins (for reviews see [1,2]). Receptor kinase most probably represents an integral part of the signal-transduction pathway as evidenced by a total loss of insulin action in cells transfected with kinase-defective mutant insulin receptors [3,4]. However, it remains unclear if the tyrosine kinase activity alone can account for all physiological actions of insulin [5,6]. Furthermore, no phosphoproteins mediating the link between the receptor kinase and the diverse effector systems have been identified until now.

Many receptors which lack the tyrosine kinase signalling mechanism have now been recognized to be coupled to their effector systems by a family of heterotrimeric guanine-nucleotide-binding proteins (G-proteins) (for a review, see [7]). Convincing evidence has been presented by Houslay and co-workers [8–10] for a G-protein-mediated regulation of cyclic AMP content by insulin in the liver. Much less information is at present available concerning the involvement of G-proteins in the regulation of glucose transport, which represents a primary action of insulin in target tissues. Kuroda et al. [11], using isolated adipocytes, suggested involvement of adenylate cyclase-related G-proteins, G_s and G_i, in glucose transport regulation by lipolytic and antilipolytic agents. More recently, Ciaraldi & Maisel [12] reported on a decreased coupling efficiency of insulin receptors in isolated adipocytes treated with pertussis toxin, whereas Luttrell et al. [13] observed a strong inhibition of insulin-stimulated hexose uptake by pertussis-toxin treatment of BC3H-1 myocytes. The latter finding has not been confirmed in isolated adipocytes [14,15]. However, recent data by Schürmann et al. [16] indicate that guanine nucleotides may modulate the activity of the insulin-sensitive glucose transporter reconstituted from membrane fractions.

Rat cardiac ventricle contains one pertussis-toxin-sensitive and three cholera-toxin-sensitive G-proteins [17]. Documented functions include regulation of adenylate cyclase [7], direct activation of Ca^{2+} channels [18] and inhibition of Na^+ channels [19]. Involvement of these transducing elements in any of the multiple cardiac insulin actions and in the regulation of the cardiac glucose transporter has not been studied before. Using isolated adult rat cardiac myocytes, our laboratory has delineated several molecular pathways of insulin action in this target tissue [20–23]. The present investigation was initiated with three aims in mind: (1) to study the involvement of G-proteins in insulin action on glucose transport, (2) to explore the effect of isoprenaline on the cardiac glucose transporter, and (3) to study the effects of diabetes on G-protein-regulated functions. The data suggest G-protein-mediated regulation of the glucose transporter by insulin and isoprenaline.

MATERIALS AND METHODS

Chemicals

3-O-[^{14}C]Methyl-D-glucose (sp. radioactivity 57.2 Ci/mol) and L-[1-^{14}C]glucose (sp. radioactivity 58 Ci/mol) were from Amersham, Braunschweig, Germany. Cyclic AMP ^{125}I radioimmunoassay kit (sp. radioactivity 150 Ci/mmol) and [γ-^{32}P]ATP (sp. radioactivity 6000 Ci/mmol) were purchased from New England Nuclear, Dreieich, Germany. Pig monocomponent insulin was obtained from Novo, Bagsvaerd, Denmark. Collagenase (EC 3.4.24.3) was from Biochrom, Berlin, Germany. BSA (fraction V, and fraction V, fatty-acid-free) was supplied by Boehringer, Mannheim, Germany. Hyaluronidase (EC 3.2.1.35), isoprenaline, theophylline, cholera toxin and pertussis toxin were from Sigma, München, Germany. Oligonucleotide probes were synthesized by Promega, Madison, WI, U.S.A., and purified by h.p.l.c. All other chemicals were analytical grade and supplied by Merck, Darmstadt, Germany.

Abbreviations used: G_s and G_i, stimulatory and inhibitory guanine-nucleotide-binding regulatory proteins, respectively, of adenylate cyclase.
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Isolation of heart cells

Male Wistar rats fed *ad libitum* and weighing 280–320 g were used in all experiments. Ca^{2+}-tolerant myocytes were isolated by perfusion of the heart with collagenase as previously described by us [21]. The final cell suspension was washed three times with Hepes buffer (composition NaCl 130 mM, KCl 4.8 mM, KH_2PO_4 1.2 mM, Hepes 25 mM, glucose 5 mM, BSA 20 g/l, pH 7.4, equilibrated with O_2) and incubated in silicone-treated Erlenmeyer flasks in a rotating water-bath shaker at 37 °C. After 20 min $CaCl_2$ and $MgSO_4$ (final concns. 1 mM) were added and incubation was continued until further use. Cell numbers were determined in a Fuchs–Rosenthal chamber; cell viability was checked by determination of the percentage of rod-shaped cells and averaged 90–95 % under all incubation conditions. Cellular ATP content was monitored in trichloroacetic acid extracts by using a Luminometer 1250 (LKB) and the firefly luciferase reaction [21].

Insulin-deficient diabetes was induced by injecting (intraperitoneally) citrate-buffered streptozotocin (pH 4.5) at a dosage of 60 mg/kg body wt. Control animals received a similar injection of the vehicle alone. All animals were kept for 3 weeks on a normal rat chow and water *ad libitum*. After that time cardiac myocytes were prepared. Blood samples were collected from the vena renalis immediately before starting perfusion of the hearts and taken for analysis of plasma glucose [24] and glycosylated haemoglobin (HbA_1), which were used for verification of the diabetic state. HbA_1 was monitored by using a column separation system obtained from ISO Lab., Akron, OH, U.S.A.

Determination of cyclic AMP

Formation of cyclic AMP by cardiac myocytes was measured by incubating 5×10^4 cells in Hepes buffer in a final volume of 0.5 ml. The reaction was started by addition of 0.2 ml of preincubated cells (as indicated in the legends) and was performed at 37 °C in a rotating water-bath shaker. After 10 min the incubation was terminated by adding 2 ml of ice-cold trichloroacetic acid and extracting the cells for 30 min. After centrifugation the extract was treated with ether, then neutralized and finally freeze-dried. Cyclic AMP was then determined by radioimmunoassay. Recovery of cyclic AMP was 80 % under all incubation conditions.

3-O-Methylglucose-transport assay

All transport experiments were performed at 37 °C in Hepes buffer, pH 7.4, containing D-glucose (5 mM). The reaction was started by addition of 50 μl of the cell suspension (2×10^4 cells) to 50 μl of Hepes buffer containing 3-O-[^{14}C]methyl-D-glucose (final concn. 100 μM). The uptake process was terminated by using a stopping solution and the oil-centrifugation technique, as described in detail by us [22]. All data for sugar uptake have been corrected for simple diffusion and extracellular trapping of radioactivity, by subtracting the amount of L-[^{14}C]glucose uptake from the amount of 3-O-methylglucose uptake, and represent specific carrier-mediated transport. All experiments were carried out in triplicate; duplicate tubes containing L-[^{14}C]glucose were run in parallel to determine non-specific uptake.

All data analysis was run on an IBM Personal Computer by using Graphpad (ISI, Philadelphia, PA, U.S.A.) statistical software. Significance of reported differences was evaluated by using the null hypothesis and t statistics for paired and unpaired data respectively. Corresponding significance levels are indicated in the legends to Figures.

RNA isolation and Northern-blot analysis

Total cellular RNA was isolated by the guanidinium thiocyanate/phenol/chloroform method [25], electrophoresed on 1.2 %-agarose gels containing formaldehyde, transferred to a nylon membrane and cross-linked by u.v. irradiation. Laser-scanning densitometry of ethidium bromide-stained RNA was used to normalize the amount of RNA loaded per lane. Blots were hybridized to a 24-mer oligonucleotide specific to the insulin-regulatable glucose transporter [26]. The oligonucleotide was 5′-end-labelled to a specific radioactivity of $(4–6) \times 10^8$ d.p.m./μg by using [^{32}P]ATP and T4 kinase. After washing under high-stringency conditions, the blots were exposed to Kodak X-Omat AR film at −70 °C by using intensifying screens. The autoradiographs were quantified by laser-scanning densitometry (LKB) to determine the relative amounts of glucose-transporter mRNA.

RESULTS

Studies on insulin-stimulated glucose transport

Receptor-independent activation of GTP-binding proteins can be achieved in intact cell preparations by combined treatment with Al^{3+} and F^- [7,15]. As an initial experiment, this protocol has now been used to probe for a functional association between the cardiac glucose transporter and G-proteins. As shown in Table 1, the combination of Al^{3+} and F^-, which results in formation of the active species AlF_4^- [27], significantly increased glucose transport, leading to a decreased non-additive stimulatory action of insulin. Transport stimulation by AlF_4^- was 53 %, and corresponded to 20–30 % of the stimulatory action of insulin, which agrees with a 40 % insulin-like activity of $AlCl_3$ observed in isolated adipocytes [15].

This observation suggested the possibility of G-protein-mediated coupling of the glucose carrier to cell-surface-located receptors. Additional data to support this assumption were provided by using cholera toxin and pertussis toxin, which have been repeatedly employed for the identification of G-protein-mediated cellular functions [7]. In order to establish significant modifications of cardiac myocyte G-proteins by these agents, isoprenaline-stimulated cyclic AMP production was determined

**Table 1. Effect of NaF and $AlCl_3$ on 3-O-methylglucose transport in isolated cardiac myocytes**

Heart cells (4×10^5 cells/ml) were incubated for 30 min in the absence or presence of the indicated additions. 3-O-Methylglucose transport activity was then determined over a 10 s assay period. The data shown are mean values ± S.E.M. of four to five separate experiments: *not significantly different from control ($P > 0.05$); **significantly different from control ($P < 0.05$).

Treatment	Insulin (0.35 μM)	3-O-Methylglucose transport (pmol/10 s per 10^6 cells)	Stimulation by insulin (%)
Control	−	384 ± 73	
	+	1268 ± 96	230
NaF (18 mM)	−	472 ± 55*	
	+	1300 ± 94	175
$AlCl_3$ (15 μM)	−	368 ± 47	
	+	1333 ± 150	262
NaF + $AlCl_3$	−	586 ± 123**	
	+	1210 ± 182	106

Table 2. Effect of cholera toxin and pertussis toxin on isoprenaline-stimulated cyclic AMP production in isolated cardiac myocytes

Cardiac myocytes were incubated with theophylline (2.5 mM), cholera toxin (25 μg/ml, preactivated by incubation with dithiothreitol for 20 min), pertussis toxin (1 μg/ml), carbachol (0.1 mM) and the indicated combinations for 2 h at 37 °C. Then 5×10^4 cells were incubated with isoprenaline (0.1 mM) for 10 min in a final volume of 0.5 ml. Cyclic AMP was then extracted and determined as outlined in the Materials and methods section. Data are means $\pm$ S.E.M. (n = 3–7): *significantly different from cells treated with theophylline alone ($P < 0.05$); **not significantly different from cells treated with pertussis toxin alone ($P > 0.05$).

Condition	Cyclic AMP production (pmol/10^4 cells)		Increase (pmol/10^4 cells)
	Basal	Isoprenaline	
Control	0.56 ± 0.15	2.21 ± 0.26	1.65
Theophylline	1.19 ± 0.66	4.41 ± 0.69	3.22
Cholera toxin + theophylline	2.12 ± 0.68	7.80 ± 1.10	5.68*
Pertussis toxin	0.46 ± 0.08	2.05 ± 0.34	1.59
Carbachol	0.75 ± 0.13	0.95 ± 0.22	0.20
Pertussis toxin + carbachol	0.46 ± 0.16	1.62 ± 0.12**	1.16
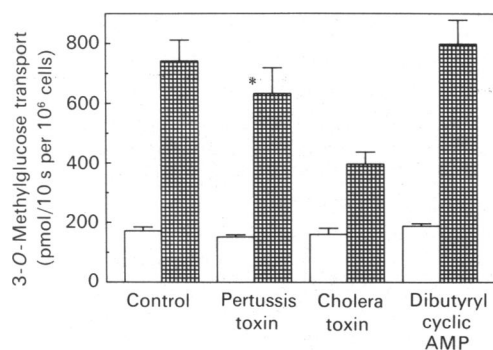
Fig. 1. Effect of pertussis toxin, cholera toxin and dibutyryl cyclic AMP on insulin action on 3-O-methylglucose transport

Cardiac myocytes (2×10^5 cells/ml) were incubated with pertussis toxin (1 μg/ml), cholera toxin (25 μg/ml) or dibutyryl cyclic AMP for 105 min. Incubation was then continued for 15 min in the absence ($\square$) or presence ($\boxplus$) of insulin (0.35 μM), followed by determination of 3-O-methylglucose transport over a 10 s assay period. The data shown are mean values $\pm$ S.E.M. obtained from four to nine separate experiments: *not significantly different from control ($P > 0.05$).

after incubation of cells with the toxins for 2 h. Cholera-toxin treatment increased both basal and isoprenaline-stimulated cyclic AMP production, with an increase of net cyclic AMP formation to 176 % of the control value (Table 2). Pertussis-toxin action was verified by testing its effect on adenylate cyclase inhibition by the muscarinic-cholinergic-receptor agonist carbachol [28]. As shown in Table 2, the inhibitory action of carbachol was substantially decreased in the presence of pertussis toxin. These data agree with the observations of Hazeki & Ui [29] in cultured adult cardiac myocytes. Viability and ATP content of treated cells were not significantly different from those of control cells [152 $\pm$ 2 nmol of ATP/10^6 cells ($n = 4$)] even after 2 h of incubation. From these findings it was concluded that under these

conditions both cholera toxin and pertussis toxin may be used to identify G-protein-mediated processes in cardiac myocytes.

As shown in Fig. 1, stimulation of 3-O-methylglucose transport by insulin was decreased from 331 % to 147 % after incubation of cells with cholera toxin, whereas pertussis toxin was without effect on transport stimulation by insulin. Basal transport rates were not affected by the toxins. The antagonistic effect of cholera toxin was not due to elevated cyclic AMP levels, since dibutyryl cyclic AMP did not alter basal and insulin-stimulated transport rates (Fig. 1). Thus at least part of insulin action on cardiac glucose transport is mediated by a cholera-toxin-sensitive G-protein.
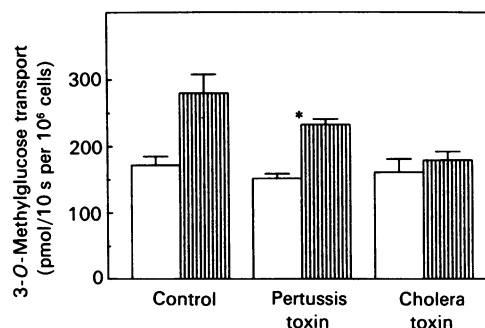
Effects of isoprenaline on glucose transport

A biphasic effect of isoprenaline on 3-O-methylglucose transport in isolated cardiac myocytes has been reported by Shanahan et al. [30], with a cyclic-AMP-independent stimulatory action of the β-agonist at concentrations above 10 nM. This finding is confirmed by the data presented in Table 3. Thus isoprenaline increases glucose transport by 63 %, an effect which is not mimicked by dibutyryl cyclic AMP and is not mediated by an increase in cytosolic Ca^{2+} concentrations, as shown by

Table 3. Effect of isoprenaline and dibutyryl cAMP on 3-O-methylglucose transport

Cardiac myocytes were incubated for 30 min in the absence or presence of the indicated additions, followed by determination of 3-O-methylglucose transport over a 10 s assay period. Data reported are mean values $\pm$ S.E.M. of three separate experiments: *significantly different from control ($P < 0.05$).

Treatment	3-O-methylglucose transport (pmol/10 s per 10^6 cells)	Stimulation (%)
Control	172 ± 13	–
Isoprenaline (0.1 mM)	280 ± 28*	63
Dibutyryl cyclic AMP (2 mM)	188 ± 8	9
KCl (50 mM)	197 ± 46	15
KCl + dibutyryl cyclic AMP	190 ± 23	10

Fig. 2. Effect of pertussis toxin and cholera toxin on isoprenaline-stimulated 3-O-methylglucose transport

Cardiac myocytes (2×10^5 cells/ml) were incubated with pertussis toxin (1 μM/ml) or cholera toxin (25 μM/ml) for 120 min in the absence ($\square$) or presence ($\boxplus$) of isoprenaline (100 μM). Glucose transport activity was then determined over a 10 s assay period. Data are means $\pm$ S.E.M. of four to nine different experiments: *not significantly different from control ($P > 0.05$).

depolarization of the cells with KCl (Table 3). In contrast with our findings, Shanahan et al. [30] have described an inhibition of both basal and insulin-stimulated glucose transport by dibutyryl cyclic AMP. However, these studies have been performed in the absence of physiological Ca^{2+} concentrations. More recently, both basal and insulin-stimulated glucose transport in cardiomyocytes has been shown to be independent of cyclic AMP [31]. It is noteworthy that under our incubation conditions dibutyryl cyclic AMP decreases insulin binding by 52 % (results not shown).

As presented in Fig. 2, stimulation of 3-O-methylglucose transport by isoprenaline is completely lost in cells treated with cholera toxin, whereas pertussis toxin is ineffective. These data suggest that both insulin receptors and β-adrenergic receptors couple to the glucose transporter by a cholera-toxin-sensitive pathway involving one or several G-proteins.

Effects of experimental diabetes

Insulin-deficient diabetes has been associated with a primary cardiomyopathy involving biochemical and functional abnormalities [32]. Furthermore, changes in expression and function of guanine-nucleotide-binding proteins [33] and a large decrease in the expression of the insulin-regulatable glucose transporter [34] have been observed in fat and muscle tissue from diabetic animals. Cardiac myocytes from streptozotocin-diabetic rats [32] have therefore been used to study possible changes of cholera-toxin-sensitive regulation of glucose transport.

As presented in Fig. 3, transport stimulation by insulin was decreased by 64 % in cardiac myocytes from streptozotocin-diabetic animals. This loss of insulin action is comparable with the effect observed in cholera-toxin-treated control cells (Figs. 1 and 3). However, in contrast with the control situation, in cells from diabetic animals insulin-stimulated transport rates were not affected by cholera toxin (Fig. 3). Thus at least 50 % of insulin action on cardiac glucose transport appears to be mediated by a cholera-toxin-sensitive G-protein. Isoprenaline action on glucose transport was completely lost in cells from diabetic rats (results not shown).

Decreased insulin action observed in diabetes appears to be related to the loss of the cholera-toxin-sensitive pathway. This may involve the glucose transporter, guanine-nucleotide-binding proteins, and additional signalling elements, including the receptor. One potential defective site has been identified by analysing the expression of the mRNA for the insulin-regulatable glucose transporter. This transporter species is exclusively
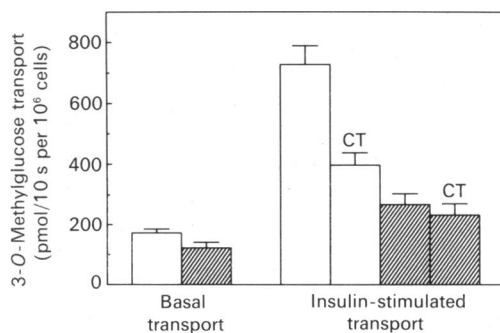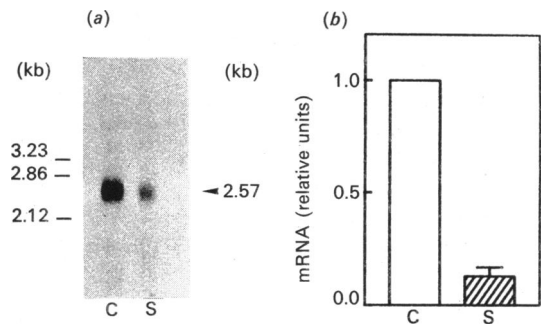

Fig. 4. Northern-blot analysis of insulin-regulatable glucose-transporter mRNA expressed in cardiac myocytes from control (C) and streptozotocin-diabetic (S) rats

(a) Total RNA was isolated, electrophoresed (10 μg/lane) on a denaturing 1.2%-agarose gel, transferred to a nylon membrane, and hybridized with a ^{32}P-labelled oligodeoxynucleotide probe complementary to the insulin-regulatable glucose transporter. Autoradiograms were exposed for 2 days. The position of RNA size markers is indicated on the left. (b) 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 four separate experiments.

expressed in insulin-responsive tissues and represents the major glucose transporter expressed in cardiac tissue [26]. Northern-blot analysis of glucose transporter mRNA showed a single transcript of 2.6 kb for both control and diabetic animals (Fig. 4a). Densitometric analysis of autoradiograms revealed a decrease in transcript concentration of the insulin-regulatable glucose transporter by 87 ± 4 % ($n = 4$) in cells from diabetic animals (Fig. 4b). These data are in perfect agreement with the results of Sivitz et al. [34], who observed an 89 % decrease in transporter mRNA in isolated adipocytes.

DISCUSSION

It is well established that insulin represents an important regulator of a variety of certain key metabolic functions of the heart [20–24], with glucose-transport stimulation being the most prominent action of this hormone. As in other target tissues, the molecular nature of cardiac transmembrane signalling by insulin remains poorly understood. Recently, tyrosine phosphorylation of G-proteins by the insulin-receptor kinase has been reported [35–37], possibly priming the transducer proteins for coupling to certain effector systems. Furthermore, tyrosine kinase-independent interaction of insulin with G-proteins has been observed in the liver [38]. Thus G-proteins may represent the missing link between the insulin receptor and the final cellular event [8–10], or vice versa, G-proteins may enable the glucose transporter to couple to several receptor systems. In the present study, this hypothesis has been evaluated in intact adult cardiac myocytes by investigating the effects of insulin and isoprenaline on glucose transport under conditions defined to recognize G-protein-mediated reactions.

Identification of G-protein-mediated functions in intact cells has largely relied on the use of cholera toxin and pertussis toxin [7], and several laboratories have successfully used this approach for elucidating a functional relationship between the glucose transporter and G-proteins [12–15,39]. The present findings confirm the notion that G-protein(s) may be part of the insulin-response signalling system in target tissues [12,13,15] and extend it to the heart muscle. Furthermore, it is shown here that G-protein-mediated activation of the glucose transporter is not restricted to the insulin receptor, and may also be achieved by β-

Fig. 3. Effect of streptozotocin-diabetes on basal and insulin-stimulated 3-O-methylglucose transport

Glucose transport activity was determined as described in the legend to Fig. 1. Where indicated, the cells were treated with cholera toxin (CT) for 2 h before the transport assay. Data are means ± s.e.m. of at least four separate experiments. Key: □, control; ■, diabetes.

adrenergic stimulation of the cardiac cell. It remains to be shown if this is brought about by one single G-protein or by several still unknown G-protein species.

Certainly, it has to be kept in mind that experiments using bacterial toxins may only provide indirect information [40] concerning the involvement of G-proteins in insulin action, and may not serve as a direct proof. However, they are strongly suggestive of such a mechanism in the cardiac cell for the following reasons: (a) toxin-mediated modulation of established G-protein functions in cardiac myocytes is documented under the same incubation conditions, (b) toxin sensitivity of glucose-transport activation is not due to non-specific perturbations of the cell, since it is not observed in myocytes from diabetic animals (see below), which are even more sensitive to metabolic alterations, and (c) toxin-sensitive reactions in intact cells have been shown to be G-protein-mediated in purified preparations in a large variety of studies [7,40].

Considerable differences have been reported concerning the effects of cholera toxin and pertussis toxin on glucose-transport activation in different tissues [12–15,39]. Thus in the muscle cell line BC_3H-1 complete blockade of insulin-induced generation of myristoyldiacylglycerol and partial inhibition of insulin-stimulated hexose uptake by pertussis toxin has been observed [13]. This would be consistent with G-protein-coupled production of insulin mediator by activation of a phospholipase C [41]. However, maximal transport stimulation has been shown to be unaffected by pertussis toxin [12,14,15] in isolated adipocytes and, as shown in the present work, in cardiomyocytes. Pertussis toxin has been reported to decrease the coupling efficiency of occupied insulin receptors to the glucose transporter [12]. This would be consistent with an effect of this toxin on insulin-receptor kinase, which has been observed in rat hepatoma cells [42]. Putting together these observations, it may be speculated that pertussis-toxin-sensitive G-proteins are more indirectly involved in glucose-transport regulation by either modulating the insulin-receptor kinase or regulating insulin mediator production. This view is supported by recent data of Burdett et al. [43], who described a G-protein-mediated regulation of the insulin-receptor kinase in L6 muscle cells.

In the present investigation cholera toxin was found to decrease maximally insulin-stimulated transport rates in cardiac myocytes to about one-half. This contrasts with the data of Ciaraldi & Maisel [12], who reported that this toxin had no influence on basal and insulin-stimulated glucose transport in isolated adipocytes. On the other hand, cholera toxin has been shown to modify insulin signalling in the liver [8], indicating a significant tissue variability in the coupling reactions [12]. One novel observation reported here is the simultaneous effect of cholera toxin on glucose-transport activation by isoprenaline. Taking into account that a large number of receptors is known to be coupled to a few G-proteins and effector systems [7], it is tempting to speculate that the glucose transporter, at least in the cardiac cell, is functionally associated with a cholera-toxin-sensitive G-protein, which mediates regulation by both insulin and β-agonists. This would involve direct G-protein–glucose-transporter coupling, and would be consistent with an effect of insulin on intrinsic carrier activity, which has been shown to occur in addition to translocation after stimulation by insulin [44].

ADP-ribosylation of G_s α-subunit by cholera toxin is known to stabilize the active species of this G-protein [7], resulting in an increased activity of adenylate cyclase [29]. It has been proposed that G_s may also interact with the glucose transporter [11]. However, basal transport rates remained unaffected in cardiomyocytes treated with cholera toxin, despite a significant increase in cyclic AMP production. Furthermore, isoprenaline

action on glucose transport was lost, although G_s-mediated activation of adenylate cyclase could still be observed. Thus it seems more likely that other, yet unidentified, G-protein species [10] may be involved. The same conclusion was reached by Schürmann et al. [16] in their studies on glucose transporter reconstituted from adipocyte membrane fractions.

Isoprenaline action on glucose transport and the cholera-toxin-sensitive part of insulin action were completely lost in myocytes from insulin-deficient animals, resulting in a decrease in insulin action to about one-third. We therefore conclude that decreased insulin action is related to this cholera-toxin-sensitive pathway and that alterations of one or several components may be responsible for the pathogenesis of insulin resistance. We show here that decreased insulin action and the loss of cholera-toxin sensitivity correlates with a much decreased expression of the insulin-regulatable glucose transporter. Since this transporter mediates the major portion [45] of insulin action, we conclude that the insulin-regulatable glucose transporter is functionally associated with a cholera-toxin-sensitive G-protein. Certainly, at the present stage we cannot rule out that insulin-deficient diabetes may also affect the expression of G-proteins, as shown by Gawler et al. [33] for the inhibitory G-protein G_i.

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