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Insulin activates GTP binding to a 40 kDa protein in fat cells

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The first steps in insulin action are binding of insulin to its receptor and activation of the insulin receptor kinase. As there is indirect evidence that further signal transduction might involve a guanine-nucleotide-binding protein (G-protein), we studied whether insulin modulates GTP binding to plasma membrane proteins of fat cells and skeletal muscle. We found that insulin rapidly increased (30 s) binding of guanosine 5′-[γ-thio]triphosphate (GTP[S]) in a dose dependent manner (0.03–2.0 nM). This effect was not altered by pertussis toxin, but it was abolished by cholera toxin treatment of fat cells. Scatchard analysis of the binding data showed that the increased GTP[S] binding is due to a decrease in the K_d for GTP from 100 nM to 50 nM. Furthermore, binding of GTP to these plasma membranes inhibited both the binding of ^{125}I-insulin to the insulin receptor and the stimulation of the insulin receptor kinase, suggesting a feedback interaction between the insulin-stimulated GTP-binding site and the insulin receptor. In order to identify this insulin-stimulated GTP-binding site, plasma membranes were labelled with the photoreactive GTP analogue [α-^{32}P]GTP γ-azidoanilide. We found that insulin selectively stimulated GTP binding to a 40 kDa protein. In conclusion, in plasma membranes of fat cells and skeletal muscle, the insulin receptor interacts with a 40 kDa GTP-binding site. We speculate that this 40 kDa GTP-binding site might be a G-protein which is involved in insulin signal transmission.

INTRODUCTION

The mechanism of cellular insulin action is not understood in detail. The early steps of insulin signal transduction involve insulin binding to the α-subunit of the receptor at the cell surface and activation of the tyrosine kinase located in the cytoplasmic part of the receptor β-subunit [1]. An intramolecular autophosphorylation cascade within the receptor kinase amplifies the insulin signal [2]. The further signal transduction to the metabolic machinery of the target cell may then occur through tyrosine phosphorylation of other substrates [3,4] or direct non-covalent interaction of the autophosphorylated receptor with regulatory proteins. Among possible candidates for these regulatory proteins are the guanine-nucleotide-binding proteins (G-proteins). A direct effect of insulin on GTP binding to one of these proteins has not been shown to date; however, there is ample indirect evidence for a role of G-proteins in cellular insulin action. There are several reports which have shown that insulin alters the ADP-ribosylation of G-proteins by pertussis toxin [5–7]. Further, a modulation of G-protein expression was demonstrated in insulin-deficient streptozotocin-diabetes [8]. There is also evidence for a role of G-proteins in insulin signalling in glucose transport, as the effect of insulin on intrinsic glucose carrier activity can be mimicked by aluminium fluoride [9], which is a known stimulator of G-proteins. A role for G-proteins in insulin signalling was furthermore suggested by the observations that G-proteins serve in a cell-free system as substrates for the purified insulin receptor kinase [10–12] and that G-proteins are able to modulate insulin receptor kinase activity [13]. This led to the speculation that a signal transfer from the insulin receptor to G-proteins might occur through phosphorylation of G-proteins at tyrosine residues [10–12]. However, other studies suggest that tyrosine phosphorylation of G-proteins is only an *in vitro* phenomenon, which is not found in intact cells [4,7]. On the other hand, it is particularly interesting that G-proteins exhibit in the cell-free system a remarkably high affinity for the autophosphorylated receptor [11]. Therefore a direct non-covalent interaction of a G-protein with the autophosphorylated receptor could possibly mediate a signal transfer from the receptor to a G-protein. We followed by hypothesis that receptor autophosphorylation might be important for such an insulin effect, and studied the binding of guanosine 5′-[γ-thio]triphosphate [GTP[S]) to plasma membranes under experimental conditions which allow the insulin receptor kinase to undergo insulin-stimulated autophosphorylation. Under these conditions we found a direct stimulatory effect of insulin on GTP binding.

EXPERIMENTAL

Materials

Pig ^{125}I-insulin (labelled on Tyr^{A14}; 2200 Ci/mmol), [γ-^{32}P]-NAD (800 Ci/mmol), GTP[^{35}S] (800–1500 Ci/mmol) and [γ-^{32}P]ATP (3000 Ci/mmol) were from New England Nuclear (Dreieich, Germany). Aprotinin, phenylmethanesulphonyl fluoride, poly(Glu$_4$:Tyr$_1$), leupeptin, pepstatin, benzamidine, bacitracin, leucine, a 5′-nucleotidase kit, cholera toxin and pertussis toxin were from Sigma (Munich, Germany). Triton X-100, all reagents for SDS/PAGE and the protein assay were from Bio-Rad (Munich, Germany). GTP and GTP[S] were obtained from Boehringer. *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodi-imide hydrochloride was purchased from Merck; Mops and 4-azidoanilline sulphate were from Serva. All other substances and reagents were of the highest grade commercially available. For autoradiography, X-Omat AR5 diagnostic film was used (Kodak, Rochester, NY, U.S.A.).

Binding of GTP[S]

Plasma membrane fractions were prepared as described previously [9]. Reproducibility of the fractionation procedure was

Abbreviations used: G-protein, guanine-nucleotide-binding protein; GTP[S], guanosine 5′-[γ-thio]triphosphate; GTP[^{35}S], guanosine 5′-[γ-[^{35}S]thio]triphosphate.

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assessed by measuring the specific 5'-nucleotidase activity in the plasma membrane fraction. To determine GTP[^{35}S] binding, plasma membrane fractions of adipocytes were used. If not otherwise stated, membrane fractions (40 μg of protein) were incubated with manganese (10 mM), ATP (200 μM) and GTP[^{35}S] (0.3 nM; 800–1500 Ci/mmol) for time intervals between 30 s and 30 min. To determine the non-specific binding of GTP[^{35}S], parallel incubations were performed in the presence of an excess of unlabelled GTP[S] (10 μM). To determine the insulin effect, 0.03–2.0 nM-insulin was added together with GTP[S]. At the end of the incubation period, membrane fractions were centrifuged at 10000 *g*. The membrane pellets were then washed and counted for radioactivity in a scintillation counter.

[^{32}P]GTP azidoanilide labelling of proteins from fat cell membranes and skeletal muscle

[α-^{32}P]GTP γ-azidoanilide was prepared as described [14]. Fat cell and skeletal muscle membranes (40 μg of protein) were preincubated for 5 min without or with insulin (2 nM) in the presence of Mn^{2+} and ATP.

[^{32}P]GTP γ-azidonanilide (3 μM) was then added for 5 min at 30 °C in the dark. Covalent coupling was then initiated by u.v. irradiation for 3 min. To determine non-specific labelling, an excess of 10 μM unlabelled GTP[S] was added. Membrane proteins were alkylated with *N*-ethylmaleimide according to Evans *et al.* [15] and applied to SDS/PAGE. The gel was exposed to Kodak X-Omat film. Labelled bands were then cut from the gel and counted for radioactivity in a scintillation counter.

Insulin binding to plasma membranes

Plasma membranes were incubated with ^{125}I-insulin (0.03 nM) and various concentrations of unlabelled insulin for 45 min at 22 °C in a medium containing 150 mM-NaCl and 25 mM-Hepes, pH 7.4. Separation of the free and receptor-bound insulin was then performed by centrifugation through dinonyl phthalate.

Determination of insulin receptor kinase activity *in vitro* and *in vivo*

Partially purified insulin receptors were prepared and phosphorylated *in vitro* as described [16]. The activation of the receptor kinase in plasma membranes was assessed by a modification of the method described for intact cells [17]. Briefly, fat cell plasma membranes were stimulated with insulin and insulin receptors were then purified as described [16], but in the presence of vanadate (1 mM). Kinase activity was measured with poly(Glu_4:Tyr_1) as substrate, as described in [16].

ADP-ribosylation

ADP-ribosylation of fat cell membranes was carried out as described [18]. Briefly, about 30 μg of plasma membranes was preincubated in the presence of insulin (2 nM) and $MnCl_2$ (10 mM) or GTP[S] (1 μM), or neither of these, for 30 min at 32 °C before ribosylation. Subsequently, ADP-ribosylation was carried out either with pertussis toxin or with cholera toxin. The reaction mixture for cholera-toxin-catalysed ribosylation contained NAD^+ (10 μM), [^{32}P]NAD^+ (5×10^6 c.p.m.), ADP-ribose (50 mM), thymidine (10 mM), ATP (1 mM), GTP (0.1 mM), EDTA (1 mM), $MgCl_2$ (10 mM), Tris/HCl (25 mM), dithiothreitol (1.66 mM) and cholera toxin (100 μg/ml) in phosphate buffer (300 mM). The reaction mixture for pertussis-toxin-catalysed ribosylation contained 10 μg of pertussis toxin/ml and the same compounds as described for cholera toxin, but without phosphate buffer, ADP-ribose and $MgCl_2$. Incubations were carried out at 32 °C for 40 min and stopped by addition of 1 ml of ice-cold 20% (v/v) trichloroacetic acid. After 20 min on ice, membranes were centrifuged at 9000 *g* for 10 min at 4 °C. The supernatants were discarded and the pellets were washed with 2×1 ml of diethyl ether. The membranes were dissolved in 30 μl of dithiothreitol (50 mM) and 5% SDS before addition of 30 μl of Laemmli sample buffer [19]. The samples were subjected to SDS/PAGE without heating on a 10% linear polyacrylamide gel. The gels were dried and exposed to Kodak X-Omat film.

RESULTS

Effect of insulin on binding of GTP[S] to plasma membranes

Hormone receptors which couple to G-proteins increase the ability of the α-subunit of the G-proteins to bind GTP [20]. Attempts to show an analogous effect for insulin have not been previously successful. Following the hypothesis that an insulin effect on a G-protein requires experimental conditions that allow the autophosphorylation of the insulin receptor kinase, we studied the properties of the binding of GTP[^{35}S] to plasma membranes of fat cells under conditions where the fat cell receptor kinase may become activated [21], i.e. in the presence of Mn^{2+} and ATP. Table 1 shows that in the absence of Mn^{2+}, in the presence or the absence of ATP, no insulin effect was detectable. In contrast, in the presence of Mn^{2+} plus ATP a significant stimulatory insulin effect was observed, whereas the effect with ATP alone was not significant. Therefore manganese and ATP were included in all further experiments. Fig. 1 shows the association kinetics of GTP[S] binding to isolated fat cell membranes. The bottom curve shows the rate of non-specific binding, which had to be subtracted from the two upper curves to obtain specific binding values. The equilibrium of specific binding was reached after 10 min. Insulin stimulated rapidly (within 30 s) the rate of association and increased equilibrium binding. Half-maximal association occurred within 2 min in the absence of insulin; in the presence of insulin, half-maximal association was reached within 1 min. Insulin increased the equilibrium binding in fat cell membranes by 54 ± 17% (mean $\pm$ S.E.M.; $n = 10$, $P < 0.005$). The same effect can be shown with skeletal muscle membranes (52 ± 23%; $n = 5$, $P < 0.005$). To determine the dose–response relationship of the effect of insulin on the binding of GTP[S], membranes were incubated with increasing concentrations of insulin (Fig. 2). The insulin effect was detectable at concentrations above 0.06 nM and reached

Table 1. Effect of insulin on GTP binding in the presence or absence of Mn^{2+} or ATP

Plasma membranes (40 μg of protein) were incubated in the presence or absence of insulin (2 nM), ATP (200 μM) and Mn^{2+} (10 mM) as indicated. GTP[^{35}S] (0.3 nM; 800–1500 Ci/mmol) was added, followed by incubation for 30 min. At the end of the incubation period plasma membranes were centrifuged at 10000 *g* and pellets were washed and counted for radioactivity in a scintillation counter. Mean values of five individual experiments ($\pm$S.E.M.) are shown. For statistical analysis, Student's *t* test was used; $^*P < 0.01$ compared with $MnCl_2$ + ATP only.

Conditions	GTP[^{35}S] binding (c.p.m.)
Basal	1031 ± 410
Insulin	1280 ± 469
Insulin + ATP	852 ± 316
$MnCl_2$ + ATP	1981 ± 247
Insulin + $MnCl_2$	2387 ± 301
Insulin + $MnCl_2$ + ATP	2926 ± 322*

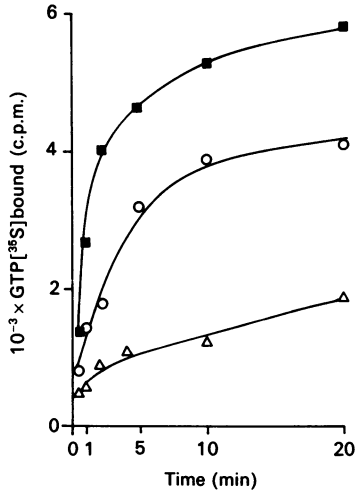

Fig. 1. Effect of insulin on the association of GTP[S] with plasma membranes

Plasma membrane fractions were prepared as described previously [13]. Protein (40 μg) was incubated with Mn^{2+} (10 mM), ATP (200 μM) and GTP[^{35}S] (0.3 nM; 800–1500 Ci/mmol) for time intervals between 30 s and 30 min. To determine the insulin effect, 2 nM-insulin was added together with GTP[^{35}S]. ○, No insulin; ■, +insulin; △, non-specific binding.

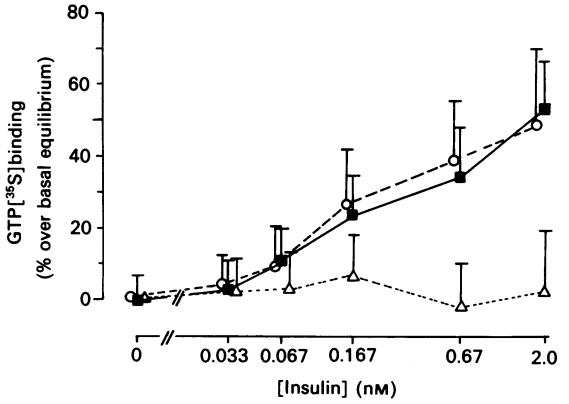

Fig. 2. Dose–response of the effect of insulin on GTP[S] binding

Fat cell membranes (40 μg of protein) were incubated with the given concentrations of insulin. Equilibrium binding of GTP[S] was then measured at 20 min with 0.3 nM-GTP[^{35}S]. The non-specific binding was determined in the presence of an excess of unlabelled GTP (10 μM) and subtracted from total binding. The values show the means ± S.E.M. of 4–10 independent experiments performed in duplicate. ■, Membranes from control fat cells; ○, membranes from fat cells which were pretreated with 0.05 μg of pertussis toxin/ml for 20 min; △, membranes from fat cells which were pretreated with 0.05 μg of cholera toxin/ml for 20 min.

its maximum at 2 nM. Thus this insulin effect had similar dose–response characteristics to those of other cellular insulin effects, such as the stimulation of glucose transport [9]. If fat cells were pretreated with pertussis toxin before preparation of membranes, no influence on the insulin effect was detected (Fig. 2). In contrast, pretreatment with cholera toxin abolished the insulin effect (Fig. 2). To determine whether the insulin effect on equilibrium binding is due to a modulation of the number or the affinity of GTP-binding sites, we performed displacement studies with increasing concentrations of unlabelled GTP[S]. Fig. 3

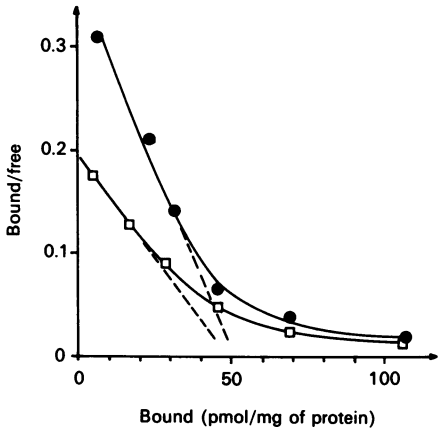

Fig. 3. Scatchard plot of GTP[^{35}S] binding to fat cell plasma membranes

Equilibrium binding of GTP[^{35}S] alone (0.3 nM) or binding in the presence of increasing concentrations of unlabelled GTP[S] was measured at 20 min. To determine the insulin effect, membrane portions were incubated with 2 nM-insulin. The non-specific binding was determined in the presence of an excess of an unlabelled GTP[S] (10 μM), and all binding values were corrected for this amount. □, Basal; ●, +insulin.

shows these data presented as a Scatchard plot. There was no effect of insulin on the number of GTP[S]-binding sites; however, insulin lowered the K_d for GTP[S] in the low concentration range by approx. 50% (50 nM to 100 nM). In summary, these data show that insulin at physiological concentrations rapidly modulates the affinity of a GTP-binding site.

Effect of GTP[S] binding to plasma membranes on insulin receptor function

The interaction of hormone receptor and G-proteins occurs often in two directions [17,22]. The hormone receptor modulates in a forward direction the ability of a G-protein to bind GTP[S]. On the other hand, GTP binding to the G-protein may exert a feedback inhibition on ligand binding and the signalling function of the hormone receptor. To investigate whether such a dual interaction exists between the insulin receptor and this GTP-binding site, we tested the effect of GTP[S] on insulin binding and on the kinase activity of the insulin receptor. Insulin binding to isolated fat cell plasma membranes is shown in Fig. 4. Preincubation of plasma membranes with GTP[S] inhibited the binding of ^{125}I-insulin predominantly at low insulin concentrations. The Scatchard plot shows that GTP[S] lowered mainly the affinity of insulin binding. To determine the effect of GTP[S] in these plasma membranes on the insulin receptor kinase, we used a modification of an earlier-described method to measure *in vivo* kinase activity [17]. Plasma membranes were stimulated with insulin, the insulin receptor kinase was then isolated under conditions where the activity state *in vivo* is preserved and kinase activity was measured *in vitro* with the synthetic substrate poly(Glu_4:Tyr_1). Preincubation of plasma membranes with GTP[S] did not alter basal kinase activity; however, it decreased the insulin-stimulated kinase activity (Table 2). To test whether this is a direct effect of GTP[S] on the insulin receptor kinase or whether the effect is mediated by the GTP-binding site described above, we studied the effect of GTP[S] on partially purified insulin receptor kinase *in vitro*. GTP[S] did not inhibit the effect of insulin on partially purified receptor kinase (results not shown), suggesting that this inhibition requires the association of the receptor with the GTP-binding protein in the plasma membrane. These data suggest that a GTP-binding site

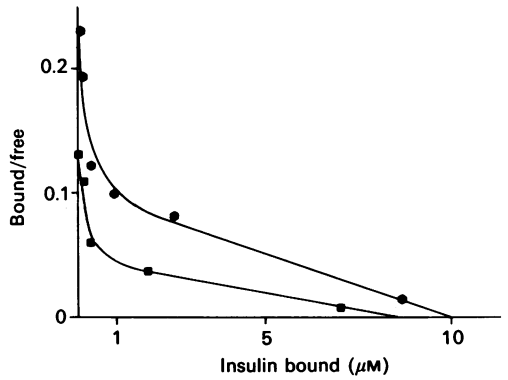

Fig. 4. Effect of GTP[S] on insulin binding

Fat cell membranes were incubated with ^{125}I-insulin (0.05 nM) and increasing concentrations of unlabelled insulin (0.05 nM–1 μM) in the presence (■) or absence (●) of GTP (10 μM). Non-specific binding was determined in the presence of 1 μM unlabelled insulin and was subtracted from all values.

Table 2. Effect of GTP[S] on insulin receptor kinase activity

Plasma membranes were stimulated with insulin (1 μM) in the absence or presence of GTP[S] (1 μM). Insulin receptor kinase was isolated in the presence of phosphatase inhibitors as described in the Experimental section and substrate kinase activity was determined *in vitro*. Mean values (% of maximum) of five individual experiments (±S.E.M.) are shown. For statistical analysis, Student's *t* test was used; $*P < 0.01$ compared with insulin treatment only.

Conditions	Receptor kinase activity (% of max.)
Basal	47.2±13.6
Insulin	99.0±9.1
Insulin+GTP[S]	76.3±4.4*

Table 3. Effect of GTP[S] on insulin receptor kinase activity after cholera toxin treatment

Fat cells were treated with cholera toxin (0.05 μg/ml) for 20 min at 37 °C. Subsequently plasma membranes were prepared and stimulated with insulin (1 μM) in the absence or presence of GTP[S] (1 μM). The insulin receptor was isolated in the presence of phosphatase inhibitors as described in the Experimental section and substrate kinase activity was determined *in vitro*. Mean values (% of maximum) of four individual experiments (±S.E.M.) are shown.

Conditions	Receptor kinase activity (% of max.)
Basal	56.8±19.2
Insulin	95.0±12.0
Insulin+GTP[S]	99.4±9.8

exists in the plasma membranes of fat cells which is activated by the insulin receptor but which is also able to inhibit insulin receptor functions, possibly by a feedback mechanism. To test whether this reverse GTP[S] effect is also cholera-toxin-sensitive, we performed the experiments described above in membranes from fat cells treated with cholera toxin. Table 3 shows that the reverse GTP effect is indeed lost after cholera toxin treatment.

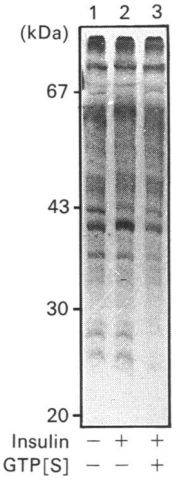

Fig. 5. Autoradiogram of [^{32}P]GTP γ-azidoanilide labelling of proteins from fat cell membranes

Fat cell membranes were preincubated for 5 min with (lanes 2 and 3) or without (lane 1) insulin (2 nM). [^{32}P]GTP azidoanilide (3 μM) was then added for 5 min at 30 °C in the dark. Covalent coupling was then initiated by u.v. irradiation for 3 min. To determine non-specific labelling, an excess of 10 μM unlabelled GTP[S] was added (lane 3).

Membrane labelling with [α-^{32}P]GTP γ-azidoanilide

To identify this insulin-sensitive GTP-binding site, we used the GTP affinity analogue [^{32}P]GTP azidoanilide [14]. Labelling with [^{32}P]GTP azidoanilide was performed in the absence of insulin, and after a 20 min preincubation with 2 nM-insulin. Fig. 5 shows an autoradiogram of the [^{32}P]GTP azidoanilide-labelled proteins from fat cell membranes. Several bands between 25 kDa and 80 kDa were labelled. Insulin increased selectively the labelling of a 40 kDa band in fat cell membranes. The same band of 40 kDa is found in membranes from skeletal muscle (results not shown). The means (±S.D.) for ^{32}P incorporation into the 40 kDa band of fat cells were 400±20 c.p.m. without insulin, 910±310 c.p.m. with insulin and 180±60 c.p.m. with insulin plus an excess of unlabelled GTP[S] ($n = 5$). As a control, bands at 24, 25, 27, 28, 35, 43 and 60 kDa were excised. The respective summarized values were 2230±530 c.p.m. without insulin, 1930±890 c.p.m. with insulin and 980±280 c.p.m. with an excess of unlabelled GTP[S] ($n = 5$), showing that insulin does not increase GTP labelling unspecifically. These data suggest that the insulin-sensitive binding site in fat cells and skeletal muscle is a 40 kDa GTP-binding protein.

ADP-ribosylation of fat cell membranes with cholera toxin and pertussis toxin

In order to test whether this protein might be a substrate for cholera toxin or pertussis toxin, we studied the effect of insulin on ADP-ribosylation of fat cell membranes. A pattern similar to that described earlier [23] for rat adipocyte plasma membranes was found. Fig. 6 shows with pertussis toxin a band of approx. 41 kDa, most likely representing the labelling of $G_i\alpha$ [23], whereas cholera toxin induced the ADP-ribosylation of bands at approx. 44–48 kDa which were previously identified as $G_s\alpha$ [23]. Pretreatment with GTP[S] had no significant effect on the pertussis-toxin-mediated ADP-ribosylation of a 41 kDa band. In contrast, the cholera-toxin-dependent ADP-ribosylation was markedly increased after preincubation with GTP[S] (without GTP[S], 165±87 c.p.m.; with GTP[S], 444±96 c.p.m.; $n = 4$). The apparent decrease in the cholera-toxin-dependent ADP-

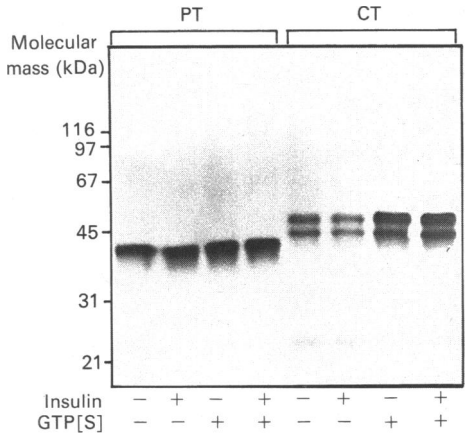

Fig. 6. ADP-ribosylation of plasma membranes

Autoradiogram of SDS/PAGE-separated proteins. Plasma membranes (30 μg of protein) from fat cells were incubated in the presence or absence of insulin (2 nM) and GTP[S] (1 μM) as indicated. After an incubation for 30 min at 32 °C, another incubation period followed with 10 μg of pertussis toxin (PT)/ml or with 100 μg of cholera toxin (CT)/ml in the presence of [^{32}P]NAD$^+$ (5×10^6 c.p.m.) for 40 min at 32 °C, as described in the Experimental section. After electrophoretic separation under denaturing conditions on a 10% polyacrylamide gel, labelled bands are detected by autoradiography.

ribosylation seen on the autoradiogram after treatment with insulin was not statistically significant (% of basal ^{32}P incorporation: 44 kDa band, 90.1 ± 20.6%; 48 kDa band, 84.2 ± 21.8%; $n = 4$). In particular, there was in four separate experiments no cholera-toxin-dependent band detectable around 40 kDa after short time exposures of the films. On overexposure of the films it could not be excluded that there existed a very faint band around 40 kDa; however, at least with the present experimental design, the existence of a cholera-toxin-ribosylated protein at 40 kDa cannot be demonstrated. There are two possible explanations for these results. First, the 40 kDa band represents a minor protein compared with other GTP-binding proteins; however, the strong labelling with the [^{32}P]GTP γ-azidoanilide argues against this explanation. Secondly, the suppression of the forward and reverse interactions between the insulin receptor and the 40 kDa protein by cholera toxin might not be due to an ADP-ribosylation of this protein by cholera toxin, but rather may reflect a direct effect of cholera toxin on the receptor. Such an effect was recently described by others [24].

DISCUSSION

Physiological concentrations of insulin stimulate GTP binding to plasma membranes with a time course similar to those of other rapid cellular insulin effects. The forward effect from the insulin receptor to a GTP-binding site is pertussis-toxin-insensitive but cholera-toxin-sensitive. A reverse effect from a GTP-binding site to the insulin receptor, i.e. the inhibition of receptor binding and kinase activation through GTP binding to plasma membranes, can also be demonstrated. The labelling experiments showed that the observed GTP binding probably occurs at a 40 kDa membrane protein. In summary, the interaction of the insulin receptor with the GTP-binding site follows classical patterns of hormone receptor and G-protein interaction [25–27]. Therefore we speculate that this 40 kDa protein might represent a specific G-protein which is involved in insulin signal transmission. At present the data do not allow us to identify the nature of this insulin-dependent G-protein. The molecular mass of a G-protein that is believed to be important in insulin action is 25 kDa [6], clearly separating this putative G_{ins} from the GTP-binding protein found here. On the other hand, the molecular mass of the protein described here is close to that of $G_i\alpha$ [22,28], $G_o\alpha$ [22,28], $G_t\alpha 1$ [29,30] and $G_t\alpha 2$ [31] and another, insulin-receptor-associated, but as yet unidentified, G-protein [32]. However, the difference in the susceptibility to pertussis and cholera toxins argues against these possible candidates. Further studies with specific antibodies are required to evaluate if this insulin-dependent G-protein is identical with one of those proteins. Concerning the role of this G-protein in insulin action, an interaction with phospholipase seems possible. There is increasing evidence that some insulin effects involve the activation of phospholipase C [33,34] and the release of inositol phosphate oligosaccharides from membrane glycolipids [35,36]. Further studies are needed to evaluate whether this insulin-dependent G-protein could serve as a link between the receptor and these phospholipases.

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