GTP-binding proteins may stimulate insulin biosynthesis in rat pancreatic islets by enhancing the signal-recognition-particle-dependent translocation of the insulin mRNA poly-/mono-some complex to the endoplasmic reticulum

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We aimed to elucidate the putative role of GTP-binding proteins in the regulation of insulin biosynthesis. For this purpose, freshly isolated rat islets were incubated in the presence of liposomes containing GDP, guanosine 5'-[β -thio]diphosphate (GDP[S]), GTP, guanosine 5'-[γ -thio]triphosphate (GTP[S]), guanosine 5'-[β -methylene]triphosphate (p[CH₂]ppG), guanosine 5'-[β -imido]triphosphate (p[NH]ppG) and ATP, and the effects of the liposomal delivery of these substances on rates of biosynthesis of insulin and total protein were determined. Insulin biosynthesis during a 1 h incubation at 1.67 mM-glucose was stimulated by ATP- and GTP[S]-containing liposomes as compared with control liposomes. At 16.7 mM-glucose, only the GTP[S]-containing liposomes stimulated insulin biosynthesis. No inhibition of islet protein and insulin synthesis was observed with GDP-, GDP[S]-, p[CH₂]ppG- and p[NH]ppG-containing liposomes. By determining the subcellular distribution of insulin mRNA, it was found that the mRNA content associated with microsomes was increased and that associated with the cytosolic mono-/poly-somes decreased when the islets were incubated with GTP[S]-containing liposomes, resulting in an approximate doubling of the ratio of microsomal to polysomal-associated insulin mRNA. ATP-containing liposomes produced no effects on the association of insulin mRNA with microsomes. By using photoaffinity labelling and immunoprecipitation techniques, specific binding of GTP[³⁵S] to the α -subunit of the signal-recognition particle (SRP) receptor in islet homogenates containing physiological concentrations of GTP and GDP was demonstrated. These findings suggest that the GTP-binding subunit(s) of the SRP receptor, and possibly also of other GTP-binding proteins involved in this process, may regulate insulin biosynthesis by stimulating the translocation of insulin mRNA to the endoplasmic reticulum and by increasing preproinsulin-peptide translocation into the lumen of the reticulum.

INTRODUCTION

The synthesis of proinsulin is a multi-step process involving such events as the association between insulin mRNA and the ribosome, leading to the formation of the initiation complex, binding of the signal-recognition particle (SRP) to the emerging signal sequence, which induces a translational inhibition and, thirdly, the translocation of the insulin-mRNA-associated poly-/mono-somes from the cytosol to the endoplasmic reticulum (ER) [1]. Finally, peptide elongation at the ER membranes ensues, a process which is regulated by glucose at low concentrations and does not involve a subcellular redistribution of insulin mRNA [1]. The association of the insulin-mRNA-poly-/mono-some complex with the ER is facilitated by the interaction between SRP and the SRP receptor, an event which leads to a dissociation of SRP from the signal sequence, release of translational arrest and translocation of the nascent peptide into the lumen of the ER [2]. In previous studies, we have studied the effects of different stimulators of insulin biosynthesis on the subcellular distribution of insulin mRNA [3]. The results obtained suggested a stimulatory action of nutrients on both the initiation of insulin biosynthesis and translocation of the insulin-mRNA-associated poly-/mono-somes to the ER membranes, the latter finding implicating the SRP/SRP-receptor interaction as a regulatory event in the biosynthesis of insulin. This effect on the SRP/SRP-receptor interaction was found to be specific for the

nutrients studied, because a non-nutrient stimulator of insulin biosynthesis, theophylline, which increases the β -cell content of cyclic AMP, enhanced initiation rates only [3]. More recently, it has been reported that the α -subunit of the SRP receptor, also called the docking protein binds GTP, and that this binding is necessary for the release of SRP from the nascent peptide and translocation of the peptide across the ER [4]. In addition, sequence information on the 54 kDa subunit of SRP [5,6] suggests that this polypeptide contains a GTP-binding domain. In view of these findings, we have introduced a non-hydrolysable GTP analogue, guanosine 5'-[γ -thio]triphosphate (GTP[S]), into the cells of isolated pancreatic islets by means of the liposome technique and studied the effects on insulin biosynthesis and the subcellular distribution of the insulin mRNA. We propose that the nutrient-stimulated insulin biosynthesis is mediated, at least in part, by the activation of GTP-binding proteins (G-proteins), particularly those involved in protein translocation across the ER membrane.

MATERIALS AND METHODS

Materials

1- α -Phosphatidylethanolamine (type III), cholesterol, oleic acid, glyoxal, poly(A), polyvinylpyrrolidone, salmon sperm DNA, dithiothreitol, Dowex MR-3, ATP (disodium salt) and Hepes were from Sigma Chemical Co., St. Louis, MO, U.S.A.

Abbreviations used: SRP, signal-recognition particle; GTP[S], guanosine 5'-[γ -thio]triphosphate; GDP[S], guanosine 5'-[β -thio]diphosphate; p[NH]ppG, guanosine 5'-[β -imido]triphosphate; p[CH₂]ppG, guanosine 5'-[β -methylene]triphosphate; ER, endoplasmic reticulum; PMA, 4-phorbol 12-myristate 13-acetate; G-protein, GTP-binding protein; NRS, normal rabbit serum; KRBH, Krebs-Ringer bicarbonate buffer + 10 mM-Hepes.

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GeneScreen hybridization transfer membrane and guanosine 5'-[γ - ^{35}S]thio]triphosphate (GTP[^{35}S]; 1300 Ci/mmol) were obtained from New England Nuclear, Boston, MA, U.S.A. Ficoll 400 and CNBr-activated Sepharose 4B were from Pharmacia Fine Chemicals, Uppsala, Sweden. L-[4,5- ^3H]Leucine (sp. radioactivity 130 Ci/mmol), [α - ^{32}P]dCTP (3000 Ci/mmol) and multiprime labelling reactions were supplied by Amersham International (Amersham, Bucks., U.K.). Crystalline mouse insulin and ^{125}I -labelled insulin were provided by Novo (Copenhagen, Denmark). Collagenase (EC 3.4.24.3), GTP[S] (tetralithium salt), guanosine 5'-[β -thio]diphosphate (GDP[S]) (trilithium salt), guanosine 5'-[$\beta\gamma$ -imido]triphosphate (p[NH]ppG) (tetralithium salt), guanosine 5'-[$\beta\gamma$ -methylene]triphosphate (p[CH $_2$]ppG) (tetralithium salt), GDP (dilithium salt) and GTP (trilithium salt) were obtained from Boehringer Mannheim (Mannheim, Germany), and Hanks' balanced salt solution was supplied by Statens Bakteriologiska Laboratorium (Stockholm, Sweden). All other chemicals of analytical grade were obtained from E. Merck (Darmstadt, Germany).

Preparation of isolated islets

Islets were isolated on Ficoll gradients [7] from collagenase-digested pancreata [8] of male Sprague-Dawley rats. The islets were picked free from other pancreatic tissue and maintained in Hanks' salt solution until further use.

Synthesis of liposomes

Phosphatidylethanolamine, cholesterol and oleic acid (molar proportions 7:7:3) were suspended in diethyl ether (2 mmol of lipid/ml), and 2 ml of the suspension was transferred to a round-bottomed retort. The diethyl ether was evaporated under a stream of nitrogen, and 0.5 ml of an aqueous solution was added. For the different liposomes, the aqueous solutions were as follows: control liposomes, 50 mM-NaCl; ATP liposomes, 50 mM-ATP; lithium liposomes, 200 mM-LiCl; GDP and GDP[S] liposomes contained 15 mM, and GTP, GTP[S], p[CH $_2$]ppG and p[NH]ppG liposomes all contained 50 mM. All solutions also contained 10 mM-Tris, pH 8.0. The lipid film was disrupted by sonication for 2 min in a sonication bath, and the resulting lipid solution was passed through a 0.2 μm -pore Millipore filter and concentrated by centrifugation at 160000 g for 10 min (4 °C). The liposomes were suspended in 50 mM-NaCl/10 mM-Tris, pH 8.0, and used within 1 h for experiments. The amount of liposomes was measured by determination of the phospholipid contents by the method described by Hallberg [9] and the encapsulation volume was assessed by using [^3H]sucrose as an aqueous-space marker. The ATP content of the liposomes was determined as described by Welsh & Sjöholm [10].

Insulin biosynthesis

Freshly isolated islets were incubated in groups of 10 at 37 °C for 60 min in 100 μl of Krebs-Ringer bicarbonate buffer [11] + 10 mM-Hepes (KRBH) containing 1.67 mM- or 16.7 mM-glucose, 0.5 mg of BSA/ml and 5 μCi of [^3H]leucine. To the islets were also added liposomes (0.3 mM lipid) containing the different solutes given in Tables 1 and 2. The islets were then washed with ice-cold buffer and subsequently sonicated in 200 μl of redistilled water. The homogenates were analysed for the amount of [^3H]leucine incorporated into (pro)insulin, by an immunoprecipitation technique [12], and total protein-biosynthesis rates were quantified by precipitation with 10% (w/v) trichloroacetic acid.

To assess the rate of hydrolysis of intraliposomal GTP[S], GTP[^{35}S] was encapsulated within liposomes, and samples were taken from the media and the islets at different time points during a typical experiment. The liposomes were disrupted by

sonication in cold 0.3 M-HClO $_4$, and GTP[S] was separated from GDP on polyethyleneimine-cellulose plates (Merck) by using 0.5 M-K $_2$ HPO $_4$ containing 10 mM- β -mercaptoethanol as solvent. The GTP[S] spots were scraped off and the radioactivity was determined by scintillation counting. The obtained values were compared with the amount of total radioactivity before the t.l.c. separation procedure. In a separate series of experiments, freshly isolated rat islets in groups of 10 were incubated in 1.67 mM- or 16.7 mM-glucose as described above with or without the addition of 100 nM-4-phorbol 12-myristate 13-acetate (PMA). Biosynthesis rates for (pro)insulin and total protein were subsequently determined as described above.

Insulin release

Groups of 10 fresh islets were incubated in duplicates at 37 °C and pH 7.4 in an atmosphere of air/5% CO $_2$. The incubation medium consisted of 100 μl of KRBH containing 0.5 mg of BSA/ml, 1.67 mM- or 16.7 mM- glucose and the different liposomes (0.3 mM lipid) as given in Table 3. The insulin concentrations in samples of the incubation media were determined by radioimmunoassay, with crystalline mouse insulin as standard and ^{125}I -insulin as a tracer [13].

Subcellular fractionation

The procedure used for subcellular fractionation was similar to that described by Welsh *et al.* [1], Bag & Sarkar [14] and Ramsey & Steele [15], but modified to compensate for differences in g force and migration distance in an Beckman Airfuge rotor. Groups of 200 freshly isolated islets were incubated for 60 min in 200 μl of KRBH containing 0.5 mg of BSA/ml, 1.67 mM- or 16.7 mM-glucose and liposomes (0.3 mM lipid) as given in Table 4. After the incubation period, the islets were washed and homogenized in 200 μl of ice-cold homogenization buffer (250 mM-sucrose, 250 mM-KCl, 10 mM-MgCl $_2$, 10 mM-Tris, pH 7.5, 1 mM-EDTA, 5 mM-dithiothreitol and 10 units of RNasin/ml) with 15 strokes in a glass tissue homogenizer. After an initial 15 s centrifugation at 12000 g to pellet nuclei and larger aggregates, the supernatant was centrifuged for 5 min at 160000 g in an Airfuge rotor. The pellet thus obtained contained insulin mRNA associated with membranes. The supernatant was further centrifuged for 2 h at 160000 g to pellet the 80 S monoribosomes. Nucleic acid was purified from all fractions by extraction with phenol/chloroform/3-methylbutan-1-ol (25:24:1, by vol.) and was precipitated with ethanol and salt from the aqueous phase.

Insulin mRNA

This was determined in subcellular fractions by dot-blot analysis [16,17]. For this purpose, pellets were treated with 1 M-glyoxal for 1 h at 50 °C, diluted with 4 vol. of 20 $\times$ SSC (SSC = 0.15 M-NaCl/0.015 M-sodium citrate) and bound to Genescreen Membranes at 80 °C for 2 h. After prehybridization, the samples were hybridized at 42 °C with the appropriate probe (pRI-7) [18], which had been labelled with [^{32}P]dCTP. After hybridization, the filters were washed and then exposed at -70 °C to Hyperfilm MP. The intensities of the spots thus obtained were determined by densitometry and expressed as optical density (o.d.).

Photo-affinity labelling of the SRP-receptor α -subunit

For this, 3000 islets were suspended in 175 μl of 0.1 M-potassium acetate/20 mM-Hepes (pH 7.0) 5 mM-magnesium acetate containing 1 mM-phenylmethanesulphonyl fluoride and 100 k-units of Trasylol/ml, and subsequently homogenized in a glass tissue homogenizer at 4 °C. The homogenate was centrifuged for 10 s at 12000 g , and the resulting supernatant was divided into three 50 μl portions to which were added 7.5 μM -GTP[S] and 70 μCi of GTP[^{35}S]. To one of the samples were also

Table 1. Effects of liposomes containing ATP and GTP[S] on islet insulin and total protein biosynthesis

Freshly isolated rat islets were incubated for 60 min in KRBH with [^3H]leucine in the presence of liposomes containing 50 mM-NaCl, 200 mM-LiCl, 50 mM-ATP or 50 mM-GTP[S] at 1.67 mM- or 16.7 mM-glucose. The rate of (pro)insulin (PI-I) biosynthesis was estimated by measuring the incorporation of [^3H]leucine into immunoprecipitated (pro)insulin. Total protein-biosynthesis rates were determined by precipitation with trichloroacetic acid (TCA). Values are means $\pm$ S.E.M. for 4–6 experiments: * $P < 0.05$ compared with LiCl; and $\dagger P < 0.05$ compared with saline, by Student's paired t test.

Liposome preparation	1.67 mM-glucose		16.7 mM-glucose	
	PI-I (c.p.m./h per islet)	(PI-I/TCA) $\times$ 100 (%)	PI-I (c.p.m./h per islet)	(PI-I/TCA) $\times$ 100 (%)
Saline	44 $\pm$ 10.2 (6)	7.5 $\pm$ 0.8 (6)	436 $\pm$ 63.2 (6)	33.5 $\pm$ 2.7 (6)
Lithium	44 $\pm$ 7.2 (6)	5.9 $\pm$ 1.1 (6)	460 $\pm$ 123.2 (6)	31.5 $\pm$ 4.5 (6)
ATP	108 $\pm$ 19.4 (4) $\dagger$	10.7 $\pm$ 1.0 (4) $\dagger$	432 $\pm$ 80.0 (4)	29.5 $\pm$ 1.7 (4)
GTP[S]	94 $\pm$ 17 (6)*	12.1 $\pm$ 2.4 (6)*	676 $\pm$ 105.2 (6)*	41.1 $\pm$ 4.1 (6)*

Fig. 1. Photo-affinity labelling of the SRP-receptor α -subunit to GTP[S]

Isolated rat islets were homogenized and incubated with 7.5 μM -GTP[S] (sp. radioactivity 200 Ci/mmol). The homogenates were then exposed to u.v. light and immunoprecipitated. The precipitates were electrophoresed on SDS/polyacrylamide gels and analysed by autoradiography. In lane 1, the homogenate was precipitated with NRS. In lanes 2 and 3, monoclonals against the SRP-receptor α -subunit were used. In lane 3, GTP (0.36 mM) and GDP (0.20 mM) were present during the incubation period. Arrowhead indicates the 70 kDa protein in lanes 2 and 3.

added 0.36 mM-GTP and 0.20 mM-GDP. The samples were kept for 5 min at room temperature, diluted to 200 μl and then exposed for 20 min to short-wave u.v. light (800 $\mu\text{W}/\text{cm}^2$) at 0–4 $^{\circ}\text{C}$. To the samples were added 250 μl of immunoprecipitation buffer (150 mM-NaCl/1% Triton X-100/10 mM-Tris, pH 7.5) and 30 μl of Protein A-Sepharose, and the mixtures were then centrifuged for 5 min at 12000 g . To one of the supernatants 1 μl of normal rabbit serum (NRS) was added, and to the other two 1 μl each of a polyclonal antiserum raised against a 27 kDa fragment of the docking-protein molecule [19], and the monoclonal antibody 12B4, which also recognized the docking protein [19], were added. After a 60 min incubation period, a mouse IgG anti-rabbit antibody was added and the incubation continued for another 60 min. The immunocomplexes were precipitated with Protein A-Sepharose (30 min), and the Sepharose was washed three times with the immunoprecipitation buffer before boiling for 5 min in 2% SDS/0.1 M- β -mercaptoethanol/0.1 M-Tris, pH 7.5. The samples were loaded on 7.5%-acrylamide/0.2%-NN'-methylenebisacrylamide/SDS gels [20] and electrophoresed at constant current (10 mA). The gels were soaked in Amplify for 20 min, dried and covered with Hyperfilm-MP at -70°C for 2 weeks.

RESULTS

Liposome characteristics

Liposomes containing ATP were characterized by measurements of encapsulation volumes, phospholipid content and ATP content. The ATP content of the liposomes corresponded well to the estimated 50 mM, and the increased

phosphate content of the ATP liposomes further substantiated the encapsulation of ATP at this concentration. To exclude the possibility that the putative effects of GTP[S] to some extent were due to the cation (lithium) of the salt, liposomes containing 200 mM-LiCl were used as a control. All liposomes were extruded through 0.2 μm Millipore filters, which ensures uniform size distributions [21]. There was no significant hydrolysis of intraliposomal GTP[S] during the 1 h experimental period as assessed by t.l.c. analysis. In a typical experiment, the recovered proportion of unhydrolysed GTP[S] in liposomes of the media was 71% at the start of the incubation and 67% after 60 min. The corresponding values for liposomes associated with the islets were 66% and 59%, respectively.

Insulin biosynthesis and release

The proinsulin biosynthesis was stimulated by both ATP and GTP[S] liposomes about 2-fold at 1.67 mM-glucose (Table 1). (Pro)insulin biosynthesis as a percentage of total protein biosynthesis was also significantly stimulated (Table 1), whereas no significant increase in total protein biosynthesis was observed (results not shown). At 16.7 mM-glucose the ATP liposomes exerted no stimulatory effect, whereas the GTP[S] liposomes enhanced (pro)insulin-biosynthesis rates, leading to an increased rate of (pro)insulin/total protein. Also at 16.7 mM-glucose there were no effects of the different liposomes on total protein synthesis. Although the (pro)insulin-biosynthesis rates were doubled at the low glucose concentration, the rates still remained far below those obtained at 16.7 mM-glucose. No effect of lithium liposomes was observed as compared with liposomes containing saline only.

To islets was added liposomally encapsulated GDP, GDP[S], GTP and its stable analogues p[CH₂]ppG and p[NH]ppG, and biosynthesis rates for insulin and total protein were determined in a separate series of experiments. The different nucleotides exerted no effects on insulin-biosynthesis rates (Table 2). There was a slight and non-significant tendency towards higher protein biosynthesis in the p[NH]ppG group at 16.7 mM-glucose. No difference in the ratios of insulin/total protein biosynthesis were observed between the different groups.

The insulin biosynthesis at 1.67 mM-glucose was 148 $\pm$ 22 (d.p.m./h per 10 islets) ($n = 4$) in control islets and 142 $\pm$ 14 in islets treated with 100 nM-PMA. The corresponding values obtained at 16.7 mM-glucose were 1200 $\pm$ 228 ($n = 4$) and 1390 $\pm$ 330 ($P > 0.05$). PMA had no effect on total protein biosynthesis (results not shown).

Islets incubated with GTP[S] liposomes at 1.67 mM-glucose released more insulin than the control islets (lithium liposomes) (Table 3). No significant effect of the ATP liposomes was,

Table 2. Effects of liposomes containing GTP, GDP and their analogues on islet insulin and total protein biosynthesis

Freshly isolated rat islets were incubated for 60 min in the presence of liposomes containing saline only (control liposomes), 15 mM-GDP, 15 mM-GDP[S], 50 mM-GTP, 50 mM-p[CH₂]ppG or 50 mM-p[NN]ppG at 1.67 mM- or 16.7 mM-glucose. The rate of (pro)insulin (PI-I) biosynthesis was estimated by measuring the incorporation of [³H]leucine into immunoprecipitated (pro)insulin. Total protein-biosynthesis rates (TCA) were determined by precipitation with trichloroacetic acid. Values are means $\pm$ S.E.M. for 6 experiments.

Liposomes	[Glucose] (mM)...	PI-I (d.p.m./h per 10 islets)		TCA (d.p.m./h per 10 islets)		(PI-P/TCA) $\times$ 100 (%)	
		1.67	16.7	1.67	16.7	1.67	16.7
Control		1180 $\pm$ 260	4370 $\pm$ 630	10700 $\pm$ 2800	15900 $\pm$ 1480	12.8 $\pm$ 3.4	27.3 $\pm$ 2.7
GDP		1030 $\pm$ 270	3880 $\pm$ 800	10300 $\pm$ 2340	15400 $\pm$ 1450	10.7 $\pm$ 3.4	24.2 $\pm$ 3.4
GDP[S]		990 $\pm$ 290	4000 $\pm$ 480	7880 $\pm$ 820	15900 $\pm$ 1860	12.1 $\pm$ 3.2	25.9 $\pm$ 2.3
GTP		1040 $\pm$ 270	3770 $\pm$ 580	10500 $\pm$ 1730	17700 $\pm$ 2760	9.5 $\pm$ 2.4	22.3 $\pm$ 2.5
p[CH ₂]ppG		1090 $\pm$ 270	3990 $\pm$ 460	9850 $\pm$ 1190	17400 $\pm$ 2010	10.4 $\pm$ 2.1	24.7 $\pm$ 1.4
p[NH]ppG		880 $\pm$ 280	4460 $\pm$ 520	9130 $\pm$ 870	19100 $\pm$ 2430	10.5 $\pm$ 3.9	24.3 $\pm$ 2.1

Table 3. Effects of ATP- and GTP[S]-containing liposomes on islet insulin release

Freshly isolated rat islets were incubated for 60 min in the presence of liposomes containing 50 mM-NaCl, 200 mM-LiCl, 50 mM-ATP or 50 mM-GTP[S] at 1.67 mM- or 16.7 mM-glucose. The insulin concentration in the incubation media were determined by radioimmunoassay, and the insulin release was expressed as ng of insulin/h per 10 islets. Values are means $\pm$ S.E.M. for 6 experiments: * P < 0.05 and ** P < 0.01 compared with 200 mM-LiCl by Student's paired t test.

Liposome preparation	Insulin release (ng/h per 10 islets)	
	1.67 mM-glucose	16.7 mM-glucose
Saline	5.6 $\pm$ 0.8	32.6 $\pm$ 8.9
Lithium	6.4 $\pm$ 0.8	44.6 $\pm$ 10.9
ATP	7.2 $\pm$ 1.3	41.9 $\pm$ 17.0
GTP[S]	8.9 $\pm$ 1.0**	84.5 $\pm$ 19.6*

however, observed. The insulin release by islets incubated with the different liposomes was clearly stimulated by the high glucose concentration. This stimulation was more pronounced with the GTP[S] liposomes than with the lithium liposomes. Again, there was no effect of lithium liposomes compared with saline only.

Subcellular distribution of insulin mRNA

As in our previous reports [1,3], more insulin mRNA was associated with the microsomal fraction and less with the poly-/mono-somal and the cytosolic fractions after incubation at 16.7 mM-glucose compared with 1.67 mM-glucose (Table 4). However, these groups were not tested statistically, since they were not performed in parallel experiments. In addition, GTP[S] liposomes further increased the amount of insulin mRNA in the microsomal fraction and decreased the amount in the poly-/mono-somal fraction at 16.7 mM-glucose. Expressed as a ratio of microsomal to polysomal-associated insulin mRNA, GTP[S] liposomes evoked almost 2-fold increases at both the low and the high glucose concentration. GTP[S] liposomes exerted no effects on the fraction of free insulin mRNA. In three separate experiments, no effects were observed with ATP-containing liposomes on the subcellular distribution of insulin mRNA (results not shown).

Photo-affinity labelling of SRP-receptor α -subunit

Electrophoresis of islet homogenates incubated with GTP[³⁵S], cross-linked and immunoprecipitated with antibodies recognizing the 72 kDa SRP-receptor α -subunit showed in four separate experiments a 70 kDa band which did not appear in the normal rabbit serum (NRS) lane (Fig. 1). The band, although fainter, was also present in the group supplemented with 0.36 mM-GTP and 0.20 mM-GDP. The proteins precipitated by NRS were also present in the other two lanes. In addition, there were also two or three high-molecular-mass bands precipitated by the anti-

Table 4. Subcellular distribution of insulin mRNA in islets incubated with liposomes containing GTP[S]

Freshly isolated rat islets were incubated for 60 min in the presence of liposomes containing either 50 mM-GTP[S] or 200 mM-LiCl at 1.67 mM- and 16.7 mM-glucose. After homogenization and subcellular fractionation of the islets, the subcellular distribution of insulin mRNA between the microsomal, free polysomal/monosomal and free cytosolic fractions was determined by dot-blot analysis. The values are optical densities (o.d.) of the insulin mRNA in each fraction determined by densitometry, and are given as means $\pm$ S.E.M. for 5–6 experiments. * P < 0.05 and ** P < 0.01 compared with lithium control by paired Student's t test.

Subcellular fraction	Insulin mRNA (o.d.)			
	1.67 mM-Glucose		16.7 mM-Glucose	
	Lithium	GTP[S]	Lithium	GTP[S]
Microsomal insulin mRNA	1.8 $\pm$ 0.4	3.0 $\pm$ 0.6	2.6 $\pm$ 0.4	3.1 $\pm$ 0.4*
Poly-/mono-somal insulin mRNA	1.6 $\pm$ 0.3	1.8 $\pm$ 0.5	1.2 $\pm$ 0.2	0.9 $\pm$ 0.2*
Free insulin mRNA	3.8 $\pm$ 1.0	4.0 $\pm$ 1.0	3.3 $\pm$ 1.6	3.4 $\pm$ 1.3
Ratio microsomal/polysomal	1.3 $\pm$ 0.1	2.1 $\pm$ 0.2**	2.7 $\pm$ 0.9	5.1 $\pm$ 2.1*

SRP-receptor antibodies which were not present in the NRS lane. These bands may represent u.v.-induced aggregates of proteins containing the 72 kDa subunit.

DISCUSSION

GTP-binding proteins (G-proteins) are involved in several cellular processes such as signal transduction at the cell membrane [22], during the initiation, elongation and membrane translocation of protein biosynthesis [23], and intracellular transport of proteins [24]. Common to all G-proteins is that they contain a highly conserved motif to which GTP binds, and that this event leads to activation of the protein, a process which further allows signal transduction. Unfortunately, nucleotides such as GTP do not transverse the plasma membrane unless artificially introduced. We have previously shown that liposomes can be utilized for this purpose, and that the functional behaviour of the β -cell can be altered if the liposomes contain biologically active molecules [25]. Liposomes are taken up mainly by endocytosis and are considered harmless to the cells [26]. This method therefore appears to have advantage over traditional permeabilization techniques, because non-permeant substances can be introduced into β -cells without damaging the cell membrane or disturbing the internal environment. More recently, we showed that this technique can be improved in islet research by using pH-sensitive liposomes [27]. This newly developed form of liposomes mediates a more efficient release of the intraliposomal contents to the cell cytoplasm, owing to destabilization of the liposomal membrane occurring on encounter with the acidic endosomes of the cell [28]. Unfortunately, it is not feasible, in intact isolated islets, to determine accurately the intracellular concentration of the liposomally delivered solute, since there is no way to discriminate between liposomes adhering to the islets and liposomes which have released their contents intracellularly. However, using islet cell suspensions and the fluorescent probe 6-carboxyfluorescein, we previously found that the intracellular release of liposomal contents corresponded to approx. 0.03 % of the cell volume during a 10 min period [27]. If this result is extrapolated to intact and freshly isolated islets and to liposomes containing a solute at 50 mM, it follows that the average intracellular concentration of the solute reaches 15 μ M after 10 min. The validity of this extrapolation is, however, somewhat uncertain. On the one hand, it may be that islet mantle cells take up the bulk of the liposomes, and the core cells (β -cells) take up only a small fraction. Therefore the effects on β -cells observed here could conceivably be mediated by the mantle cells. On the other hand, freshly isolated islets may have open intercellular spaces, which allow small liposomes (50–200 nm in diameter) to penetrate. In addition, the extracellular matrix of trypsin-treated islets cells may have been enzymically damaged, leading to a decreased ability of the islet cells to endocytose liposomes. In favour of the latter interpretations are our previous findings that intact islets take up more liposomes than do free islet-cell suspensions [25], that the proportion of carboxyfluorescein-positive cells was not higher in islet-cell suspensions than in intact islets [27], and that glucose-6-phosphate-containing pH-sensitive liposomes stimulate insulin release and biosynthesis in intact islets, an effect probably not mediated by mantle cells [29]. In view of these findings, it is plausible to assume that our assessment of the liposomal delivery to islet β -cells is not overestimated and that the effects observed in the present work indeed reflect direct actions on the β -cell. However, since it was also found that the extent of the release of liposomal contents was not uniform among the islets cells [27], the intracellular

concentration of the nucleotides introduced in the present work may have varied considerably between the individual islet β -cells.

The role of G-proteins in stimulating the activities of adenylate cyclase and phospholipase C is now well established [22]. Thus the stimulatory effect of GTP[S], which is known to bind and activate G-proteins, observed in the present work on insulin release may reflect increased rates of generation of cyclic AMP, InsP₃ and diacylglycerol, all intracellular messengers implicated in glucose-stimulated insulin release. In addition, using permeabilized RINm5F cells, Vallar *et al.* [30] showed that G-proteins may exert stimulatory effects on the release of insulin by other mechanisms which do not involve the above-mentioned messengers. The stimulatory effects of GTP[S] contrast with the failure of ATP to affect insulin secretion. Closure of the ATP-sensitive K⁺ channel, which leads to depolarization, Ca²⁺ influx and exocytosis [31], plays a crucial role in the regulation of insulin secretion. Since the activity of the K⁺ channel is thought to be regulated by the cytoplasmic ATP/ADP ratio, the lack of effect of the ATP-containing liposomes was somewhat surprising. It should be noted that the rate of liposomal ATP delivery is almost insignificant as compared with the very high islet rate of total ATP synthesis, and it could therefore be envisaged that the liposome-delivered ATP was insufficient to close the channel. Surprisingly, and contrary to this, the ATP-containing liposomes stimulated the rates of insulin biosynthesis. This suggests that ATP may be a regulator (directly or indirectly) of insulin biosynthesis as well [32]. The effect of ATP on insulin biosynthesis occurred without a concomitant redistribution of insulin mRNA, a finding a very reminiscent of the effects of low glucose concentrations on insulin-biosynthesis rates [1], thus suggesting that ATP may primarily regulate the elongation of proinsulin. Despite the low contribution of liposomal ATP to total islet ATP generation, the observed stimulatory effects on insulin biosynthesis may be explained by intracellular delivery of the liposomal ATP to a very limited subcellular compartment in which insulin biosynthesis takes place.

The GTP analogues p[NH]ppG and p[CH₂]ppG are more resistant to hydrolysis than is GTP[S], but these analogues commonly exhibit considerably lower affinity for G-proteins than does GTP[S] [33–38]. Therefore it is not surprising that the more stable analogues did not mimic the GTP[S] action observed in the present work by stimulating insulin biosynthesis. Non-hydrolysable GTP analogues are known to inhibit protein synthesis *in vitro* by preventing the elongation and translocation steps [39]. We have detected an inhibitory action of GTP[S] in a system for translation *in vitro* (results not shown); however, the effect was modest at the GTP[S] concentrations corresponding to the estimated concentrations delivered by liposomes *in vivo* (30 % inhibition of protein synthesis at 15 μ M-GTP[S]). In contrast, no such inhibitory effect was observed when using liposomes and intact islets, which points to differences between systems *in vivo* and *in vitro*. The reason for this discrepancy is obscure; however, a putative decrease in protein synthesis may be masked by the activation of other G-proteins leading to a stimulated adenylate cyclase activity and an increased production of cyclic AMP, a well-known stimulator of protein biosynthesis in pancreatic islets [3]. Furthermore, different G-proteins may show different relative affinities for GTP[S], and thus the elongation system of intact islets may be more resistant to GTP[S] inhibition than the system *in vitro*. Nevertheless, it is likely that the conditions *in vivo* used here allowed specific and significant binding of GTP[S] to the SRP receptor, since we could demonstrate that this binding occurred *in vitro*, under conditions resembling the situation *in vivo*, that is, at the estimated concentration of delivered GTP[S] and at physiological concentrations of GTP and GDP [40].

The recent findings that the SRP-receptor α -subunit is a G-protein and that subunits of the SRP also may bind GTP [4–6] has led to the hypothesis that the sorting of secretory proteins to the ER is a GTP-dependent process. A biological rationale for this mechanism was given by comparing with the function of the bacterial Tu protein [41]. This G-protein 'proof-reads' tRNA molecules simultaneously with GTP hydrolysis, and thereby prevents misincorporation of amino acids into nascent proteins. By analogy with this, the G-proteins of the SRP and the SRP receptor may 'proof-read' the signal sequence and ensure a high fidelity in sorting proteins to the correct destinations. Unlike elongation, the sorting and translocation process does not require hydrolysis of GTP [4]. In addition to this putative role in protein biosynthesis, our present findings suggest that the G-proteins of the SRP and the SRP receptor may function as regulatory sites in the translational control of insulin biosynthesis. The GTP/GDP ratio is considerably lower in the pancreatic β -cell than in most other cell types: 13 in liver cells, compared with 2–4 in the β -cell [40]. Thus this ratio may, in the insulin-producing cell, be closely regulated and especially suitable for controlling the activity of G-proteins. Since the SRP receptor appears to possess a higher affinity for GDP than for GTP [4], any GTP/GDP ratio of 2 or less will result in a significant proportion of the SRP receptor being in a GDP-bound and inactivate state. Since glucose increases the GTP/GDP ratio to about 4 [40], it is quite tempting to propose that glucose, by increasing the GTP/GDP ratio, stimulates the translocation of insulin mRNA from the cytosol to the ER and the translocation of the nascent peptide into the lumen of the ER, and thereby in part regulates the rate by which insulin is synthesized. Our experimental results with the GTP[S]-containing liposomes favour this hypothesis, since GTP[S] stimulated insulin biosynthesis and translocation of the insulin mRNA to microsomes in a way similar to that of glucose. Even though the intracellular GTP[S] content is low in the present system, it is high enough to bind the SRP receptor significantly, even in the presence of the intracellular GTP and GDP concentrations. It may be argued that the redistribution of insulin mRNA observed in the present work could be due to an increased content of cyclic AMP or a stimulated protein kinase C activity. However, in a previous study, we showed that the phosphodiesterase inhibitor theophylline did not enhance the association of insulin mRNA to microsomes [3]. This finding, in conjunction with the present observations that protein kinase C activation did not lead to stimulated insulin biosynthesis, suggests that activation of the cyclic AMP and the protein kinase C systems does not directly induce translocation of insulin mRNA.

As a consequence of our proposal that the GTP/GDP ratio serves a role in regulating the SRP-dependent process of targeting and translocating across the ER membrane, other proteins synthesized at the ER with signal sequences should also be positively regulated by glucose. For some, this is the case [42,43]. However, heterogeneity in signal-sequence-SRP interactions [6,44] may explain the inability of glucose to stimulate the synthesis of carboxypeptidase H [43], thus excluding this protein and probably others from the proposed scheme.

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