Characterization of the solubilized glibenclamide receptor in a hamster pancreatic β -cell line, HIT T15

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The glibenclamide receptor, a putative ATP-sensitive K^+ channel in the hamster pancreatic β -cell line HIT T15, was solubilized by using the zwitterionic detergent CHAPS. [3H]Glibenclamide binding was dependent on the incubation time and on the concentration of soluble membrane protein. Over 80% of [3H]glibenclamide bound could be displaced with 1 μM non-labelled glibenclamide. The curve relating specific binding to the concentration of [3H]glibenclamide (1–20 nM) showed saturation kinetics. Scatchard analysis suggested a single class of non-interacting binding sites with a K_d of 3.3 nM and a B_{max} of 90 fmol/mg of protein. [3H]Glibenclamide binding to solubilized membranes was inhibited by glibenclamide, tolbutamide and meglitinide. The relative potency of these agents on binding of [3H]glibenclamide to solubilized membranes was similar to that observed with microsomal preparations and paralleled their effects on K-ATP channel activity, measured as ^{86}Rb efflux. These data show that the sulphonylurea receptor in the pancreatic β -cell can be solubilized in an active form retaining specificity for sulphonylureas. ADP, which inhibits [3H]glibenclamide binding to microsomal preparations or intact HIT β -cells, did not inhibit binding to the solubilized receptor. Incubation of intact HIT β -cells with ^{125}I -glibenclamide derivative followed by exposure to u.v. light resulted in covalent labelling of a peptide of 65 kDa on SDS/PAGE. The extent of labelling increased with ^{125}I -glibenclamide derivative concentration (1–20 nM) and was inhibited in the presence of excess unlabelled glibenclamide.

INTRODUCTION

K^+ channels inhibited by intracellular ATP (K-ATP channels), originally described in heart (Noma, 1983), are now known to occur widely and provide a way to couple metabolic activity to plasma-membrane electrical activity. In the pancreatic β -cell, K-ATP channels play a crucial role in stimulus–secretion coupling (for a review see Ashcroft & Ashcroft, 1990). These channels participate in the control of insulin release by glucose (Ashcroft *et al.*, 1984) and may also be involved in the modulation of insulin release by hormones, including vasopressin (Martin *et al.*, 1989), galanin (de Weille *et al.*, 1988) or somatostatin (Fosset *et al.*, 1988). The effect of glucose is mediated by intracellular metabolism of the sugar and involves changes in intracellular [ATP]/[ADP] ratio. Sulphonylureas such as glibenclamide, used clinically in the treatment of non-insulin-dependent diabetes mellitus, also inhibit, by a direct action, K-ATP channels in islet cells (Sturgess *et al.*, 1985) or β -cell lines such as RIN m5F (Schmid-Antomarchi *et al.*, 1987) or HIT T15 (Niki *et al.*, 1989b). Closure of the channel causes depolarization of the β -cell membrane, resulting in an increase of Ca^{2+} influx through the voltage-dependent Ca^{2+} channel.

High-affinity binding sites for sulphonylureas have been described in β -cell membranes (Kaubisch *et al.*, 1982; Geisen *et al.*, 1985; Schmid-Antomarchi *et al.*, 1987; Gaines *et al.*, 1988; Niki *et al.*, 1989b). The relative ability of various sulphonylureas to displace [3H]glibenclamide from β -cell membranes parallels their ability to block K-ATP channels and stimulate insulin release (Panten *et al.*, 1989). It is not established, however, whether the glibenclamide receptor is itself the K-ATP channel or whether it is a separate, channel-associated, protein.

Exposure to u.v. light of β -cell membranes incubated with [3H]glibenclamide results in the covalent labelling of proteins ranging in size from 30 to 140 kDa on SDS/PAGE (Kramer

et al., 1985). A peptide of 140 kDa, photo-labelled with a ^{125}I -glibenclamide derivative, has been solubilized and partially purified (Aguilar-Bryan *et al.*, 1990). However, solubilization of the β -cell glibenclamide receptor in an active form has not been reported.

The present study aimed to solubilize active β -cell glibenclamide receptor and to compare its properties with those observed in whole cells or microsomes. We have used the cloned β -cell line HIT T15, which we have shown previously to contain K-ATP channels and high-affinity glibenclamide-binding sites (Niki *et al.*, 1989b). Some of these data have been reported in abstract form (Ashcroft & Niki, 1990).

MATERIALS AND METHODS

Materials

HIT-T15 cells were generously provided by Professor A. E. Boyd III (Houston, TX, U.S.A.). Glibenclamide, tolbutamide, meglitinide (HB699), [3H]glibenclamide and ^{125}I -glibenclamide derivative (no. 35623) were gifts from Hoechst (Frankfurt, Germany). Diazoxide was a gift from Glaxo (Greenford, Middx., U.K.). $^{86}RbCl$ was purchased from Amersham International (Amersham, Bucks., U.K.). RPMI 1640 tissue-culture medium was from Imperial Laboratories (Andover, Hants., U.K.). Foetal-calf serum, trypsin/EDTA, antibiotic and antimycotic solution, phosphate-buffered saline (Dulbecco's) and Multi-wells and flasks for tissue culture were purchased from Gibco (Uxbridge, Middx., U.K.). Glass-fibre filters (GF/F) were obtained from Whatman (Maidstone, Kent, U.K.). Sephadex G-50 (Medium) was purchased from Pharmacia (Uppsala, Sweden). Oligomycin, 2-deoxyglucose, ADP, the proteinase inhibitors, CHAPS, Triton X-100 and deoxycholate were purchased from Sigma (Poole, Dorset, U.K.). Optifluor was pur-

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chased from Packard Instruments (Downers Grove, IL, U.S.A.). Other agents used here were from BDH (Poole, Dorset, U.K.) and were of the purest grade available.

Cell culture

HIT T15 β -cells (passage numbers 80–95) were cultured in RPMI 1640 tissue-culture medium containing penicillin (100 units/ml), streptomycin (0.1 mg/ml), fungizone (0.25 μ g/ml) and foetal-calf serum (10%, v/v) at 37 °C in an atmosphere of humidified air/CO₂ (19:1) as previously described (Ashcroft *et al.*, 1986). Cells were passaged weekly and harvested with trypsin/EDTA. HIT cells were seeded in culture flasks at 5×10^7 cells per flask or 5×10^5 cells per Multi-well. Rat pancreatic islets were isolated by collagenase digestion and cultured in RPMI 1640 medium as described by Andersson (1978).

Assay of K-ATP channel activity

K-ATP channel activity was assayed by measuring glibenclamide-sensitive ⁸⁶Rb efflux (Niki *et al.*, 1989b). On the day before an experiment, ⁸⁶RbCl (sp. radioactivity: 86–682 Ci/mol) was added to wells (0.1 μ Ci/well), and cells were loaded with the radioisotope overnight. On the day of the experiment, the culture medium was removed and replaced with 1 ml of solution A [containing (mM): NaCl 124; CaCl₂ 1.8; MgCl₂ 0.8; KCl 10; Hepes 20 (pH 7.5 with NaOH)], to which was added ⁸⁶RbCl (0.1 μ Ci/ml). To deplete the cells of ATP in order to open the ATP-sensitive K⁺ channel, preincubation was carried out for 20 min at 37 °C in 1 ml of solution A containing ⁸⁶RbCl (0.1 μ Ci/ml) in the absence or presence of 1.2 μ g of oligomycin/ml and 1 mM-2-deoxy-D-glucose. This pretreatment decreased the concentration of intracellular ATP from 5 to 0.5 mM (Niki *et al.*, 1989a). After the preincubation, the medium was replaced with solution A without ⁸⁶RbCl and the cells were incubated for 10 min in the absence or presence of different concentrations of meglitinide. Alternatively, Rb efflux through the ATP-sensitive K⁺ channel was evoked by a 10 min incubation with 300 μ M-diazoxide. To determine the cellular content of ⁸⁶Rb, cells were washed twice with solution A supplemented with 1 μ M-glibenclamide (to prevent loss of ⁸⁶Rb via the ATP-sensitive K⁺ channel) and then extracted with 1 ml of 0.1 M-boric acid (pH 8 with NaOH) and 0.5 M-NaCl. The extract was counted for radioactivity by Čerenkov counting.

Membrane preparation

HIT-cell microsomes were prepared as described in Niki *et al.* (1989b). In brief, after washing with 2×20 ml of phosphate-buffered saline (Dulbecco's), trypsin-treated HIT cells (approx. 10^9 cells) were resuspended in 20 ml of a hypo-osmotic buffer containing 5 mM-Tris base (pH 8.0 with HCl) with proteinase inhibitors (1 mM-phenylmethanesulphonyl fluoride, 10 μ g of soybean trypsin inhibitor/ml, 10 μ M-leupeptin and 1 mM-iodoacetamide), left for 40 min on ice and then homogenized. The homogenate was centrifuged for 10 min at 900 g. Then the supernatant was further centrifuged for 30 min at 96000 g. The pellets were resuspended at a concentration of 1–3 mg protein/ml in a buffer containing 20 mM-Mops (pH 7.4 with NaOH) and the proteinase inhibitors described above. Collected membranes were frozen immediately in liquid nitrogen and stored at –70 °C.

Binding of [³H]glibenclamide to membranes

Collected membranes (150–250 μ g of protein/ml) were incubated in the presence of 1–3 nM-[³H]glibenclamide with or without various concentrations of non-labelled glibenclamide,

tolbutamide or meglitinide in a buffer containing 50 mM-Mops and 0.1 mM-CaCl₂. After incubation for 120 min at room temperature, the mixture was poured on to a Whatman GF/F filter soaked in the incubation buffer, followed by washing with 5×5 ml of distilled water. The filter was placed into 10 ml of aqueous/non-aqueous scintillant (Opti-Fluor), left overnight and counted for radioactivity by liquid-scintillation spectrometry.

Solubilization of HIT-cell membranes

HIT-cell membranes were solubilized by incubation at 4 °C for 60 min with gentle stirring in solution B, containing 50 mM-Tris (pH 8 with HCl), 0.2 mM-CaCl₂, 10% (v/v) glycerol, 1 mM-phenylmethanesulphonyl fluoride, 10 μ g of soybean trypsin inhibitor/ml, 10 μ M-leupeptin and 1 mM-iodoacetamide, and supplemented with 2% CHAPS. The mixture was re-centrifuged at 96000 g for 30 min to spin down any remaining particulate material, and the supernatant was retained.

Binding of [³H]glibenclamide to solubilized membranes

Solubilized HIT-cell membranes (100–600 μ g/ml) were incubated with 1–25 nM-[³H]glibenclamide and other additions as stated. At the end of the incubation, bound radioactivity was separated from free by rapid gel filtration: 2 ml or 5 ml of Sephadex G-50 (medium grade; Pharmacia) equilibrated with buffer B plus 0.1% CHAPS, was poured into a 2 ml or 5 ml plastic syringe respectively. The column was pre-spun for 4 min at 120 g. Then 180 μ l (for a 2 ml column) or 450 μ l (for a 5 ml column) of the incubation mixture was carefully loaded on to the column. The column was spun again for 4 min at 120 g. The eluate was collected and counted for radioactivity. Approx. 75% of the radioactivity bound in the sample was recovered in the eluate as compared with a conventional gel-filtration method using photo-labelled protein (see below). Non-specific binding was determined as residual binding in the presence of 1 μ M non-labelled glibenclamide. Background was determined with a parallel control without soluble materials.

Photoaffinity labelling

HIT cells were cultured in Petri dishes (2.5 cm, 5×10^5 cells/dish; or 6 cm, 2×10^6 cells/dish). After 4–6 days of culture, medium was removed. The cells were washed with phosphate-buffered saline [PBS; ionic composition (mM): Na⁺ 154, K⁺ 4, Cl[–] 140, H₂PO₄^{2–} 10; pH 7.4], and then incubated with [¹²⁵I]-glibenclamide derivative (1–20 nM; 2000 Ci/mmol) in PBS at 4 °C. After 30–60 min the cells were exposed to u.v. light (max. 280 or 302 nm) at 800 μ W/cm² for 20 min at 4 °C. The cells were lysed for 15 min on ice in 0.5% Triton X-100/0.5% sodium deoxycholate/0.1% SDS/150 mM-NaCl/20 mM-Tris (pH 7.5)/1 mM-EDTA containing 1 mM-PMSF and 150 k-units of Trasylol/ml. Lysed cell extracts were spun at 15000 g for 5 min. Acetone was added to the supernatant (final concn. 70%, v/v). The precipitated proteins were redissolved in sample buffer and resolved by discontinuous SDS/PAGE on 7.5% or 10%-acrylamide/0.2%-bisacrylamide gels (Laemmli, 1970). Gels were dried and then autoradiographed by using Hyperfilm. Batches of 500 rat islets were similarly labelled with [¹²⁵I]-glibenclamide derivative.

Other methods

Protein was determined (Bradford, 1976) with BSA as standard. Stock solutions of oligomycin (2.4 mg/ml) and glibenclamide (4 mM) were prepared in dimethyl sulphoxide. Tolbutamide and meglitinide at 100 mM were dissolved in 120 mM-NaOH, and the pH was adjusted if required.

RESULTS

Effect of meglitinide on K-ATP-channel activity

Fig. 1 shows the effect of meglitinide on ^{86}Rb efflux evoked by (a) diazoxide or (b) ATP depletion. Diazoxide increased ^{86}Rb efflux from $12.6 \pm 1.7\%$ to $42.5 \pm 1.9\%$ ($n = 8$). Increasing concentrations of meglitinide progressively inhibited the diazoxide-evoked ^{86}Rb efflux; at the highest concentration (1 mM) so far tested, ^{86}Rb efflux from the cells was the same as that without diazoxide (Fig. 1a). When K-ATP channels were opened by ATP-depleting pretreatment with oligomycin and 2-deoxyglucose (Fig. 1b), ^{86}Rb efflux was increased from $12.6 \pm 1.6\%$ for control to $55.7 \pm 3.1\%$ for ATP-depleted cells over a 10 min incubation ($n = 8$; $P < 0.001$). Addition of meglitinide less effectively inhibited the ^{86}Rb efflux caused by ATP depletion: no inhibition was observed at $1\ \mu\text{M}$, and at 1 mM the efflux was greater than that from diazoxide-treated cells ($10.5 \pm 1.8\%$ from diazoxide-treated cells versus $16.9 \pm 1.1\%$ from ATP-depleted cells; $n = 4$, $P < 0.05$).

Fig. 1. Inhibition by meglitinide of ^{86}Rb efflux from HIT cells caused by (a) diazoxide and (b) ATP depletion

(a) ^{86}Rb efflux over 10 min was expressed as a percentage of the ^{86}Rb content at the start of the incubation. Diazoxide ($300\ \mu\text{M}$) with or without various concentrations of meglitinide was added during the 10 min incubation. (b) ATP depletion was carried out by pre-exposure of the cells to $1.2\ \mu\text{g}$ of oligomycin/ml and 1 mM-2-deoxyglucose for 20 min. Data are given as means $\pm$ S.E.M. for 4–8 observations.

Fig. 2. Time course for $[^3\text{H}]$ glibenclamide binding to solubilized HIT-cell membranes

Solubilized membranes containing $380\ \mu\text{g}$ of protein/ml were incubated with $25.3\ \text{nM}$ - $[^3\text{H}]$ glibenclamide at 4°C for 15–120 min. At the end of the incubation, bound radioactivity in the mixture was assessed by rapid gel filtration. Each point is the mean of duplicate measurements with a single membrane preparation.

Fig. 3. Protein-dependent $[^3\text{H}]$ glibenclamide binding to solubilized HIT-cell membranes

$[^3\text{H}]$ Glibenclamide ($25.3\ \text{nM}$) was incubated with solubilized HIT-cell membranes (0 – $600\ \mu\text{g}$ of protein/ml) at 4°C for 90 min. At the end of the incubation, bound radioactivity in the mixture was assessed by rapid gel filtration. Each point is the mean of duplicate measurements.

$[^3\text{H}]$ Glibenclamide binding to HIT-cell microsomes or solubilized HIT-cell membranes

Fig. 2 shows the time course of $[^3\text{H}]$ glibenclamide ($25\ \text{nM}$) binding to solubilized HIT-cell membranes. The binding reached equilibrium within 60 min, and the extent of binding was proportional to the protein concentration between 300 and $600\ \mu\text{g/ml}$ (Fig. 3).

As shown in Fig. 4(a), 1 – $22\ \text{nM}$ - $[^3\text{H}]$ glibenclamide exhibited dose-dependent and saturable binding to solubilized HIT-cell membranes. Non-specific binding was linear and less than 20% of the total binding. A representative Scatchard plot for specific binding is shown in Fig. 4(b); the plot was linear, suggesting the presence of one saturable binding site, with a K_d value of $3.3\ \text{nM}$ and a B_{max} of $90\ \text{fmol/mg}$ of protein.

In Fig. 5, the effects of non-labelled glibenclamide, tolbutamide and meglitinide on the binding of $[^3\text{H}]$ glibenclamide ($7\ \text{nM}$) to solubilized membranes are compared with their ability to displace $[^3\text{H}]$ glibenclamide from microsomes. Glibenclamide, tolbutamide and meglitinide dose-dependently inhibited $[^3\text{H}]$ glibenclamide binding both to solubilized membranes and to microsomes. Non-specific binding of $[^3\text{H}]$ glibenclamide was somewhat greater with

Fig. 4. Saturable binding of $[^3\text{H}]$ glibenclamide to solubilized HIT-cell membranes

(a) Solubilized membranes were incubated with various concentrations of $[^3\text{H}]$ glibenclamide in the presence (non-specific; $\bullet$) or absence (total; $\circ$) of $1\ \mu\text{M}$ non-labelled glibenclamide. The incubations were carried out for 90 min at 4°C . Binding activity was measured by rapid gel filtration. Each point is a single determination, but the data are representative of 3 similar experiments. (b) Data from a representative experiment are expressed as a Scatchard plot.

microsomal preparations than with solubilized membranes; 50% inhibition of specific $[^3\text{H}]$ glibenclamide binding by glibenclamide, tolbutamide and meglitinide was observed at 14 nM, 210 μM and 120 μM respectively for solubilized preparations, and at 7 nM, 55 μM and 60 μM respectively for binding to microsomes.

ADP at concentrations up to 4 mM did not displace $[^3\text{H}]$ glibenclamide from solubilized membranes (results not shown).

Photoaffinity labelling of intact HIT-cells

After SDS/PAGE of HIT-cell proteins prepared from intact cells covalently labelled with ^{125}I -glibenclamide derivative by u.v. illumination, a single radioactive species of 65 kDa was observed (Fig. 6a). The extent of labelling was dependent on the concentration of ^{125}I -glibenclamide derivative over the range 1–20 nM. The labelling of membrane protein in the presence of 5 nM derivative was inhibited by unlabelled 1–10 μM glibenclamide (Fig. 6b). Because glibenclamide is capable of covalently labelling albumin (Kramer *et al.*, 1985), we also included BSA (50 $\mu\text{g}/\text{ml}$) in control labelling experiments. However, there was no labelling of albumin under these conditions (results not shown), thus excluding the possibility that the 65 kDa protein was albumin incompletely removed from

Fig. 5. Inhibition of $[^3\text{H}]$ glibenclamide binding to HIT-cell microsomes and solubilized membranes by glibenclamide, tolbutamide and meglitinide

Solubilized HIT-cell membranes were incubated with 1–3 nM- (for microsomes; $\circ$) or 7 nM- (for solubilized receptor; $\bullet$) $[^3\text{H}]$ glibenclamide in the presence or absence of various concentrations of unlabelled glibenclamide (a), tolbutamide (b) or meglitinide (c). The incubations were carried out for 90 min at 4°C . Each point is the mean $\pm$ S.E.M. of 3–5 observations.

Fig. 6. Photolabelling of intact HIT cells or rat islets with ^{125}I -glibenclamide derivative

(a) HIT cells (5×10^6) were incubated with 1 nM-, 2 nM-, 5 nM-, 10 nM- or 20 nM- ^{125}I -glibenclamide derivative (1, 2, 5, 10, 20 respectively) or with 5 nM- ^{125}I -glibenclamide derivative + 20 μM unlabelled glibenclamide derivative (+) for 30 min at 4 °C. The cells were exposed to u.v. light for 20 min and lysed before SDS/PAGE electrophoresis and autoradiography. Alternatively, 500 pre-cultured rat islets were incubated with 5 nM- ^{125}I -glibenclamide derivative and exposed to u.v. light (is). The result is representative of 3 separate experiments. (b) The ability of unlabelled glibenclamide (0.4–10 μM) to inhibit the cross-linking of 5 nM- ^{125}I -glibenclamide derivative to intact HIT cells is shown. The data represent the area under the 66 kDa peak calculated by densitometric scanning of autoradiograms of SDS gels as described in (a).

cells during the washing procedure. When isolated rat islets were photolabelled by the ^{125}I -glibenclamide derivative, a band of identical molecular mass was detected (Fig. 6a).

DISCUSSION

Sulphonylureas have been shown to bind with high affinity to membrane fractions from pancreatic β -cells (Kaubisch *et al.*, 1982; Gaines *et al.*, 1988; Niki *et al.*, 1989b), cardiac muscle cells (Fosset *et al.*, 1988) and brain (Lupo & Bataille, 1987). The role of K-ATP channels closely related to the glibenclamide receptor is well established in the pancreatic β -cell. HIT T15 cells have previously been used to investigate and characterize K-ATP channels and have been shown to have high-affinity binding sites for glibenclamide (Gaines *et al.*, 1988; Niki *et al.*, 1989b, 1990; Aguilar-Bryan *et al.*, 1990) and K-ATP channels with a high sensitivity to sulphonylureas (Niki *et al.*, 1989b).

We have previously shown that the activity of K-ATP channels in HIT cells can be measured as the glibenclamide-inhibitable component of ^{86}Rb efflux from cells depleted of ATP or treated with diazoxide (Niki *et al.*, 1989b). The ability of tolbutamide and glibenclamide to inhibit ^{86}Rb efflux under these conditions correlated well with the ability of these sulphonylureas to compete with [^3H]glibenclamide binding to β -cell membranes and to

stimulate insulin release. In the present study we wished to extend these observations to the related drug meglitinide, which is not a sulphonylurea but which contains the non-sulphonylurea moiety of glibenclamide, and may similarly inhibit ATP-sensitive K^+ channels (Garrino *et al.*, 1985; Zünkler *et al.*, 1988; Sturgess *et al.*, 1988; Panten *et al.*, 1989). We confirmed that meglitinide inhibits HIT β -cell K-ATP channels measured in this way at concentrations that markedly decreased the binding of [^3H]glibenclamide to HIT-cell membrane preparations.

The main aim of the present study was to prepare active solubilized glibenclamide receptors. We first showed that HIT-cell membranes solubilized with CHAPS retained glibenclamide-binding activity. The estimated K_d value for solubilized receptor preparations is somewhat higher than that found in microsomes from β -cell membranes [HIT cells: 0.7 nM (Gaines *et al.*, 1988), 1.1 nM (Niki *et al.*, 1989b), 0.36 nM (Aguilar-Bryan *et al.*, 1990); RIN m5F cells: 0.3 nM (Schmid-Antomarchi *et al.*, 1987)], but is similar to the value of 3.6 nM found in whole HIT β -cells (Niki *et al.*, 1990). Low-affinity binding sites for glibenclamide, previously reported in HIT-cell microsomes (Niki *et al.*, 1989b) or brain microsomes (Lupo & Bataille, 1987), were not observed in the solubilized preparation. The B_{max} was approximately one-eighth of that estimated with membrane-binding studies using the same cell line (Niki *et al.*, 1989b). Our B_{max} , however, is underestimated, since we cannot collect all the bound radioactivity in the assay mixture with rapid gel filtration, and some binding activity may be lost during the solubilization procedure.

The relative ability of glibenclamide, tolbutamide and meglitinide to compete with the binding of [^3H]glibenclamide to solubilized membranes was similar to that found for the particulate binding activity. Moreover, the relative potency was also similar to that seen for inhibition of K-ATP channels in β -cells, measured either electrophysiologically (Sturgess *et al.*, 1988) or by inhibition of ^{86}Rb efflux (Niki *et al.*, 1989b; the present work), suggesting that the selectivity of the receptor for these substances was retained in the solubilized form.

We reported that ADP competitively inhibited [^3H]glibenclamide binding to whole HIT cells or to HIT-cell microsomes, and that the nucleotide inhibited ^{86}Rb efflux from HIT cells caused by ATP depletion or diazoxide (Niki *et al.*, 1990). However, in the present study, [^3H]glibenclamide binding to the solubilized material was not modified by ADP. The reason is unclear. It is possible that the binding between the receptor and ADP involves another mechanism, e.g. mediation by a further protein which was lost on treatment with CHAPS.

In preliminary studies we found that binding of ^{125}I -glibenclamide derivative to microsomal fractions from HIT cells displays non-saturating kinetics, suggesting non-specific labelling of proteins (see also Aguilar-Bryan *et al.*, 1990). To minimize possible non-specific labelling, we used intact HIT cells in the present study to attempt to label the glibenclamide receptor. Under these conditions a single species of 65 kDa on SDS/PAGE was observed. A similar or identical band was obtained with normal rat islet cells. The dose-dependence of the photolabelling suggested that the labelled protein had high affinity for the glibenclamide derivative. Moreover, the presence of unlabelled glibenclamide derivative decreased the photolabelling of the 65 kDa protein, suggesting specific binding. However, we consistently found that higher concentrations of glibenclamide were required to compete with ^{125}I -glibenclamide derivative for photolabelling of intact cells than were needed to displace [^3H]glibenclamide from particulate or solubilized membrane preparations; this could reflect differences in the properties of the radioactive analogues.

Previous studies using membrane preparations rather than intact cells for photolabelling with radioactive glibenclamide

have demonstrated labelling of a 140 kDa component (Kramer *et al.*, 1985; Aguilar-Bryan *et al.*, 1990). However, depending on the exact conditions of photolabelling and the labelled ligand used, the migration of the protein on SDS gels may be changed because of possible cross-linking of homologous or heterologous protein species. Thus the 140 kDa component previously observed could be a dimer of glibenclamide-binding subunits. Purification studies are required to resolve this point.

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