Volume 229, number 2, 355–359 FEB 05665 March 1988

Direct photoaffinity labeling of the putative sulfonylurea receptor in rat β-cell tumor membranes by [^{3}H]glibenclamide

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Received 25 January 1988

The oral antidiabetic sulfonylurea [^{3}H]glibenclamide specifically binds to plasma membranes from a rat β-cell tumor indicating a receptor for sulfonylureas in these membranes. Irradiation of [^{3}H]glibenclamide at 254 or 300 nm in the presence of albumin resulted in covalent labeling of the albumin molecule. Direct photoaffinity labeling of β-cell membranes with [^{3}H]glibenclamide resulted in the covalent modification of two membrane polypeptides with apparent molecular masses 140 and 33 kDa. The extent of labeling of the 140 kDa polypeptide was specifically decreased by sulfonylureas. This suggests that a membrane polypeptide of 140 kDa is a component of the sulfonylurea receptor in the β-cell membrane.

Sulfonylurea receptor; β-Cell membrane; Photoaffinity labeling; Glibenclamide

1. INTRODUCTION

Sulfonylureas are widely used in the treatment of non-insulin-dependent diabetes mellitus. The molecular mechanisms of insulin release from islets of Langerhans by the action of sulfonylureas are still not yet fully understood [1,2]. Specific binding of sulfonylureas to receptors of the β-cell membrane has been shown [3–6]. It has been suggested that tolbutamide decreases the K^+ permeability of β-cell membranes [7,8]. By patch-clamp experiments it has been shown that the hypoglycemic sulfonylureas inhibit an ATP-sensitive K^+ channel [9–15]. From these data it has been suggested that the sulfonylurea receptor may be part of an ATP-sensitive K^+ channel [9,10]. However, protein components of the sulfonylurea receptor of β-cell membranes have not yet been identified. Since the technique of photoaffinity labeling has been successfully used for the identification of membrane receptors [16–18], in the present study we have used [^{3}H]glibenclamide as a photoaffinity probe for the sulfonylurea receptor of the β-cell membrane derived from a rat tumor.

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2. MATERIALS AND METHODS

2.1. *Materials*

[^{3}H]Glibenclamide (spec. act. 31 Ci/mmol) was synthesized as in [3]. Glibenclamide and tolbutamide were from Hoechst Aktiengesellschaft (Frankfurt). Molecular mass marker proteins for electrophoresis were obtained from Sigma (München), whereas the chemicals for electrophoresis were from Serva (Heidelberg). Quickszint® 501 and Biolute® were obtained from Zinsser Analytic (Frankfurt).

2.2. *Preparation of β-cell membranes*

β-Cell membranes from the rat β-cell tumor grown in rats of the strain NEDH (New England Deaconess Hospital, obtained from S. Warren [19]) were prepared as described [3] and stored at −70°C.

2.3. *Photoaffinity labeling*

For photoaffinity labeling of albumin with [^{3}H]glibenclamide, solutions of human serum albumin (1 mg/ml) in 100 mM sodium phosphate buffer (pH 7.4) were incubated in the dark at 20°C with 0.25 μM [^{3}H]glibenclamide for 60 min. Irradiation was performed in a Rayonet RPR 100 photochemical reactor (Southern Ultraviolet Co., Hamden, CT), equipped with 16 RPR 2530, 3000 or 3500 Å lamps at a distance of 10 cm from the lamps. After definite times aliquots were removed, adjusted to 200 μl with water and protein

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00145793/88/$3.50

precipitated as in [20]. For photoaffinity labeling of β-cell membranes, membranes were thawed at 30°C and resuspended in 100 mM sodium phosphate buffer (pH 7.4). 600 μg of β-cell membranes were incubated in a total volume of 200 μl with 40–60 nM (0.3–0.4 μCi) [^{3}H]glibenclamide at 20°C in the dark for 60 min. After irradiation at 254 nm for 2 min membranes were diluted with 1 ml of 10 mM Tris-Hepes buffer (pH 7.4)/4 mM EDTA/4 mM iodoacetamide/4 mM PMSF and centrifuged at 48000 × g for 30 min. The resulting pellet was resuspended in 200 μl water and protein precipitated as in [20].

2.4. *SDS gel electrophoresis*

The dried protein precipitates were dissolved in 70 μl of 62.5 mM Tris-HCl buffer (pH 6.8)/2% SDS/5% 2-mercaptoethanol/0.005% bromophenol blue by shaking on a mixer for 60 min. After centrifugation at 15000 × g for 10 min the clear supernatants were submitted to SDS gel electrophoresis on 150 × 180 × 1.5 mm slab gels as described [21]. After fixing and staining the gels were scanned with a CD-50 densitometer (Desaga, Heidelberg), thereafter radioactivity being determined by liquid scintillation counting after slicing the gels into 2-mm pieces and after digestion of proteins with Biolute.

3. RESULTS AND DISCUSSION

The binding of [^{3}H]glibenclamide to β-cell membranes is a saturable process suggesting specific binding sites for glibenclamide in the β-cell membrane. The specific binding of [^{3}H]glibenclamide could be displaced by other sulfonylureas. It was demonstrated that the inhibition of [^{3}H]glibenclamide binding to β-cell membranes by glibenclamide, tolbutamide and other sulfonylurea derivatives in vitro correlates to the blood sugar lowering activity in vivo [3]. Since the binding of [^{3}H]glibenclamide to β-cell membranes is of high affinity and specific, it should be possible to identify the respective receptor for sulfonylureas by photoaffinity labeling [16–18]. A prerequisite for such an approach is the availability of suitable photolabile derivatives of the respective ligand molecule. Glibenclamide contains aromatic

Table 1

Photocatalyzed incorporation of [^{3}H]glibenclamide into albumin

Irradiation time (min)	Incorporation of radioactivity (dpm)	
	254 nm	300 nm
0	<30	<30
1	275	263
2	745	509
3	1089	710

Human serum albumin (1 mg/ml) in 10 mM sodium phosphate buffer (pH 7.4)/140 mM NaCl was incubated with 0.25 μM (8 μCi/mg protein) [^{3}H]glibenclamide at 20°C for 10 min in the dark. Photoaffinity labeling was carried out at 254 or 300 nm. After 0, 1, 2 and 3 min irradiation aliquots containing 40 μg protein were removed and protein was precipitated. The covalent attachment of [^{3}H]glibenclamide to albumin was determined by liquid scintillation counting after SDS gel electrophoresis and slicing of the gels

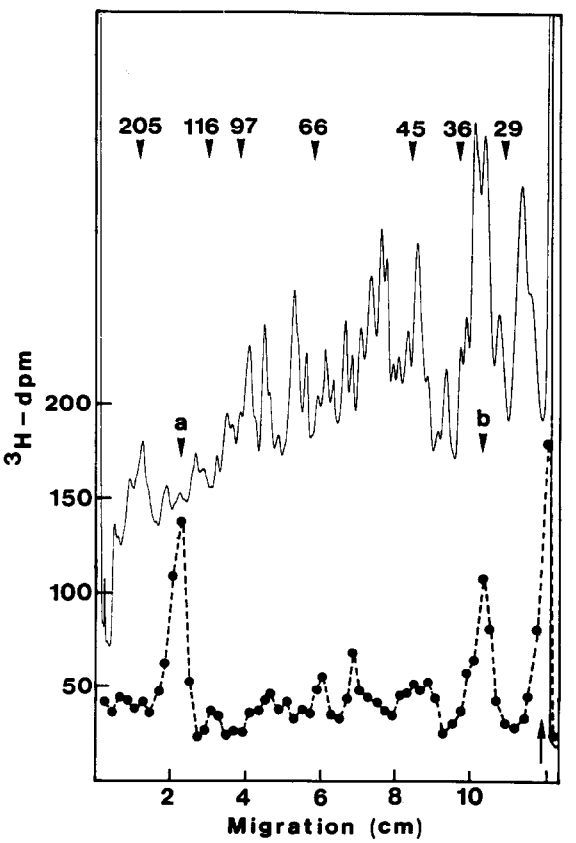

Fig.1. Distribution of radioactivity after SDS gel electrophoresis of β-cell membranes photolabeled with [^{3}H]glibenclamide. 600 μg β-cell membranes were incubated for 60 min at 20°C in the dark with 48.3 nM (0.3 μCi) [^{3}H]glibenclamide and subsequently irradiated at 254 nm for 2 min. After washing of the membranes, proteins were separated by SDS gel electrophoresis. Total acrylamide concentration was 7.5%. The continuous line shows the distribution of Serva blue R 250-stained polypeptides, the dashed line denoting the distribution of radioactivity. Molecular masses (in kDa; at top of figure) of the marker proteins and the position of the tracking dye bromophenol blue are indicated by arrows.

benzene rings and aromatic *O*-methyl ethers within the molecule comprising photoreactive structures [16,17]. The ability of [^{3}H]glibenclamide to act as a direct photoaffinity probe was investigated with human serum albumin. Albumin solutions were incubated with [^{3}H]glibenclamide and irradiated at 254, 300 or 350 nm. Subsequently, covalent incorporation of radioactivity into the albumin molecule was measured by liquid scintillation counting after denaturing SDS gel electrophoresis. Table 1 shows that during irradiation at 254 or 300 nm [^{3}H]glibenclamide was covalently attached to albumin. Therefore, [^{3}H]glibenclamide can be used for direct photoaffinity labeling of binding proteins for sulfonylureas in different tissues.

Photoaffinity labeling of β-cell membranes with [^{3}H]glibenclamide revealed clear covalent incorporation of radioactivity into two membrane polypeptides of apparent molecular masses of 140 kDa (fig.1, peak a) and 33 kDa (fig.1, peak b) after SDS gel electrophoresis. By variation of the gel concentration from 6 to 15% total acrylamide

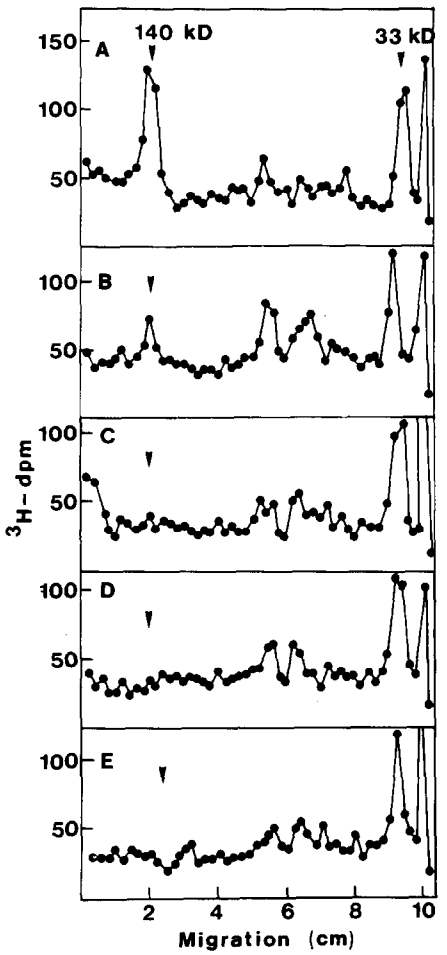

Fig.2. Influence of glibenclamide on photoaffinity labeling of β-cell membranes by [^{3}H]glibenclamide. β-Cell membranes (600 μg) were incubated for 60 min in the dark with 0 M (A), 10^{-8} M (B), 10^{-7} M (C), 10^{-6} M (D) or 10^{-5} M (E) glibenclamide. After incubation with 60 nM (0.37 μCi) [^{3}H]glibenclamide for 10 min in the dark the membranes were photolabeled at 254 nm for 2 min. After washing of the membranes the proteins were separated by SDS gel electrophoresis on 7.5% gels.

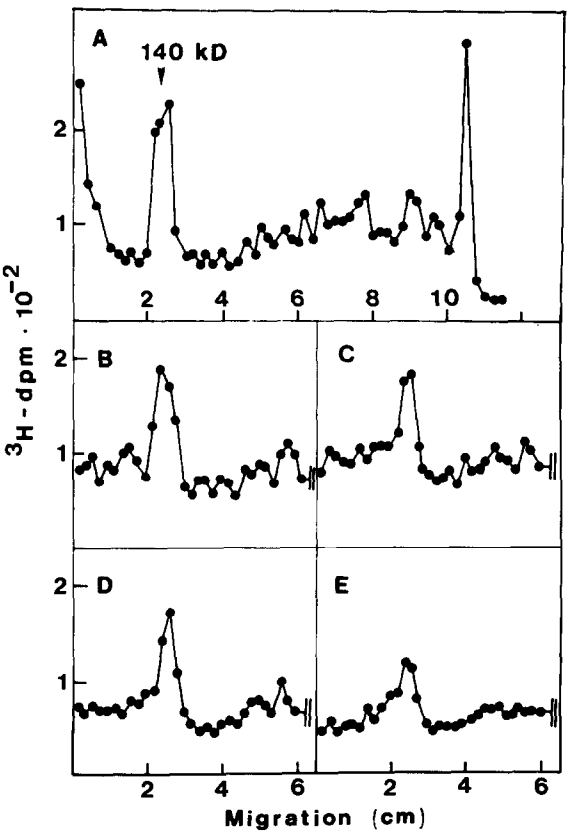

Fig.3. Influence of tolbutamide on the photoaffinity labeling of β-cell membranes with [^{3}H]glibenclamide. β-Cell membranes (600 μg) were incubated for 60 min in the dark with 0 M (A), 10^{-7} M (B), 10^{-6} M (C), 10^{-5} M (D) or 10^{-4} M (E) tolbutamide. After incubation with 58 nM (0.36 μCi) [^{3}H]glibenclamide for 10 min in the dark membranes were photolabeled at 254 nm for 2 min. After washing of the membranes the proteins were separated by SDS gel electrophoresis on 7.5% gels.

no additional labeled polypeptides of lower or higher molecular mass could be detected. Incubation of β-cell membranes with [^{3}H]glibenclamide without irradiation did not result in radiolabeling of any membrane polypeptides, demonstrating photocatalyzed incorporation of radioactivity into the above-mentioned membrane proteins. After heating of the β-cell membranes to 70°C prior to photoaffinity labeling, no radiolabeling of membrane proteins occurred. This indicates a specific interaction of [^{3}H]glibenclamide with the respective binding proteins of the β-cell membrane. Fig.1 shows that the distributions of radioactivity and of stained polypeptides are quite different demonstrating specific labeling of membrane proteins. The radiolabeled glibenclamide-binding polypeptide of 140 kDa is a minor component of the β-cell membrane.

The specificity of labeling of β-cell membrane proteins by [^{3}H]glibenclamide was further substantiated by competition labeling experiments. Sulfonylureas, like unlabeled glibenclamide or tolbutamide, clearly decreased labeling of the 140 kDa polypeptide in a concentration-dependent manner, whereas labeling of the 33 kDa polypeptide was not significantly affected (figs 2,3). Unlabeled glibenclamide was more effective than tolbutamide at decreasing the extent of labeling of the 140 kDa polypeptide. In repeated experiments a half-maximal decrease in labeling was achieved with about 2×10^{-8} M glibenclamide, whereas with 2×10^{-5} M tolbutamide a 1000-fold higher concentration of this sulfonylurea was necessary to obtain a 50% decrease in labeling of the 140 kDa polypeptide. This difference in sensitivity to photoaffinity labeling between glibenclamide and tolbutamide correlates clearly to their different affinity in binding to the membrane and to the difference in their hypoglycemic activity [3]. It has been suggested that the sulfonylurea receptor may be a K^+ channel [9–15]. The proteins of Ca^{2+} channels in various tissues have been characterized using different (photo)affinity probes and polypeptides of 140–170, 52–60 and 32–35 kDa have been identified as components of these Ca^{2+} channels [22–24]. Since the molecular masses of these Ca^{2+}-channel proteins are similar to those of the sulfonylurea-binding polypeptides from the β-cell membrane, Ca^{2+}-channel drugs were also used for competition labeling experiments. Verapamil, nifedipine or ajmaline to a concentration of up to 10^{-4} M exerted, in contrast to sulfonylureas, no significant protective effect on the labeling of [^{3}H]glibenclamide-binding proteins of the β-cell membrane (not shown).

In conclusion, for the first time a specific binding protein for sulfonylureas in the cell membrane of β-cells has been identified by photoaffinity labeling. We assume that this membrane polypeptide with an apparent molecular mass of 140 kDa is a component of the sulfonylurea receptor in the β-cell membrane.

Acknowledgements: The authors thank F. Girbig, I. Leipe and R. Saar for excellent technical assistance.

REFERENCES

[1] Lebovitz, H.E. (1985) in: The Diabetes Annual 1 (Alberti, K.G.M.M. and Krall, L.P. eds) pp.93–110, Elsevier, Amsterdam, New York.
[2] Hellmann, B. and Täljedal, J.B. (1975) in: Handbook of Experimental Pharmacology, New Series, vol.32/2, pp.175–194, Springer, Berlin.
[3] Geisen, K., Hitzel, V., Oekonomopulos, R., Pünter, J., Weyer, R. and Summ, H.-D. (1985) Arzneim.-Forsch./Drug Res. 35, 707–712.
[4] Kaubisch, N., Hammer, R., Wollheim, C., Renold, A.E. and Offord, R. (1982) Biochem. Pharmacol. 31, 1171–1174.
[5] Joost, H.G., Schwarz, P. and Göke, B. (1980) Akt. Endokrinol. Stoffw. 1, 177.
[6] Lebovitz, H.E. (1984) Diabetes Care 7, 67–71.
[7] Henquin, J.C. (1980) Diabetologia 18, 151–160.
[8] Henquin, J.C. and Meissner, H.P. (1982) Biochem. Pharmacol. 31, 1407–1415.
[9] Sturgess, N.C., Cook, D.L., Ashford, M.L.J. and Hales, C.N. (1985) Lancet 8453, 474–475.
[10] Schmid-Antomarchi, H., De Weille, J., Fosset, M. and Lazdunski, M. (1987) J. Biol. Chem. 262, 15840–15844.
[11] Ashcroft, F.M., Kakei, M., Kelly, R.P. and Sutton, R. (1987) FEBS Lett. 215, 9–12.
[12] Trube, G., Rorsman, P. and Ohno-Shosaku, T. (1986) Pflügers Arch. 407, 493–499.
[13] Arkhammar, P., Nilsson, T., Rorsman, P. and Berggren, P.-O. (1987) J. Biol. Chem. 262, 5448–5454.
[14] Schmidt-Antomarchi, H., DeWeille, J., Fosset, M. and Lazdunski, M. (1987) Biochem. Biophys. Res. Commun. 146, 21–25.
[15] Castle, N.A. and Haylett, P.G. (1987) J. Physiol. 383, 31–43.
[16] Bayley, H. (1983) in: Laboratory Reagents in Biochemistry and Molecular Biology, vol.12 (Work, T.S. and Burdon, R.H. eds) Elsevier, Amsterdam, New York.

[17] Chowdhry, V. and Westheimer, F.H. (1979) Annu. Rev. Biochem. 48, 293–325.
[18] Ruoho, A.E., Rashidbaigi, A. and Roeder, P.E. (1984) Recept. Biochem. Methodol. 1, 119–160.
[19] Chick, W.L., Warren, S., Chute, R.N., Like, N.A., Lauris, V. and Kitchen, K. (1977) Proc. Natl. Acad. Sci. USA 74, 628–632.
[20] Wessel, D. and Flügge, U.I. (1984) Anal. Biochem. 138, 141–143.
[21] Kramer, W., Burckhardt, G., Wilson, F.A. and Kurz, G. (1983) J. Biol. Chem. 258, 3623–3628.
[22] Glossmann, H.D., Ferry, A., Goll, A., Striessnig, J. and Zernig, G. (1985) Arzneim.-Forsch./Drug Res. 35, 1917–1935.
[23] Galizzi, J.-P., Borsotto, M., Barhanin, J., Fosset, M. and Lazdunski, M. (1986) J. Biol. Chem. 261, 1393–1397.
[24] Glossmann, H., Ferry, D.R., Striessnig, J., Goll, A. and Moosburger, K. (1987) Trends Pharmacol. Sci. 8, 95–105.