THE PANCREATIC β-CELL RECOGNITION OF INSULIN SECRETAGOGUES. INHIBITORY EFFECTS OF A MEMBRANE PROBE ON THE ISLET UPTAKE AND INSULIN-RELEASING ACTION OF GLIBENCLAMIDE*

Bo HELLMAN, Åke LERNMARK, Janove SEHLIN and Inge-Bert TÖLJEDAL
Department of Histology, University of Umeå, 901 87 Umeå, Sweden

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1. Introduction

Studies on the volume distribution of sulfonylureas in pancreatic islets have indicated that these drugs do not readily enter the β-cells but more likely exert a direct action on their plasma membranes [1, 2]. It seems moreover possible that the secretagogic capacity of sulfonylureas depends on their affinity for the β-cell membrane, since a much greater uptake was observed for glibenclamide than for tolbutamide, of which two drugs tolbutamide is the least active. However, this correlation may be co-incidental, and we decided to look for more direct evidence supporting a causal relationship between the islet uptake and the insulin-releasing activity of sulfonylureas. Since according to the hypothesis these drugs act on the β-cell plasma membrane, we have investigated the effects of 4-acetamido-4′-isothiocyanate-stilbene-2,2′-disulfonic acid (SITS), a compound considered to react specifically with the plasma membranes of other cells [3–5]. It will be shown that SITS markedly inhibits the uptake as well as the insulin-releasing activity of glibenclamide in the β-cell-rich pancreatic islets of *ob/ob*-mice.

2. Experimental

SITS was obtained from British Drug Houses Ltd., Poole, England. *N*-4-[2-(5-Chloro-2-methoxy-benzamido)-ethyl]-phenyl-sulfonyl-*N*′-cyclohexyl-[1-^{3}H]urea (glibenclamide) as well as non-radioactive glibenclamide were donated by Farbwerke Hoechst A.G., Frankfurt/Main, W. Germany. Adult *ob/ob*-mice were taken from the Umeå colony. Fresh pancreatic islets were microdissected freehand [6] and subsequently incubated at 37°C, using Krebs–Ringer bicarbonate buffer equilibrated with $O_2 + CO_2$ (95:5) as the basal medium. In studies of glibenclamide uptake the islets were incubated with ^{3}H-labelled glibenclamide plus either [^{14}C]urea or 3-*O*-methyl-D-[U-^{14}C]glucose. Details of the incubations including the concentrations of these compounds as well as of SITS are given in the legend to table 1. Since urea and 3-*O*-methyl-D-glucose distribute in the total islet water [7], uptake of glibenclamide in excess of the urea or 3-*O*-methyl-D-glucose space indicates that the sulfonylurea derivative is concentrated in the islets. Previous studies suggest that this excess reflects binding of glibenclamide to the β-cell plasma membranes [2]. The employed double-label procedure makes it possible to obtain a meaningful parameter of glibenclamide uptake without washing the islets after incubation in order to remove contaminating incubation fluid. However, the total content of glibenclamide in the islet samples will also be given as a check that the effects of SITS were not due to altered urea or 3-*O*-methyl-D-glucose spaces. In studies of insulin release the islets were incubated for 60 min with glibenclamide and SITS as described in the legend to table 2. Glibenclamide was added to the media from a 50 mM stock solution in dimethylformamide, resulting in a medium concentration of 0.2% (v/v) dimethylformamide. The same concentration of dimethylformamide

* Part VI of a series.

Table 1
Effect of SITS on the islet uptake of glibenclamide.

Incubation time	No of experiments	mmol Glibenclamide/kg dry wt. of islets: 0 mM SITS	1 mM SITS	Effect of SITS
Experiments with urea as space marker				
3 min	8	0.246 ± 0.021 (0.331 ± 0.022)	0.209 ± 0.018 (0.296 ± 0.019)	−0.037 ± 0.007** (−0.036 ± 0.010**)
60 min	8	0.541 ± 0.036 (0.657 ± 0.038)	0.322 ± 0.030 (0.443 ± 0.032)	−0.219 ± 0.029*** (−0.214 ± 0.028***)
Experiments with 3-*O*-methyl-D-glucose as space marker				
3 min	4	0.305 ± 0.021 (0.381 ± 0.024)	0.256 ± 0.009 (0.337 ± 0.010)	−0.050 ± 0.023 (−0.044 ± 0.024)
60 min	4	0.681 ± 0.065 (0.773 ± 0.067)	0.385 ± 0.040 (0.483 ± 0.038)	−0.296 ± 0.049** (−0.291 ± 0.055*)

After preliminary incubation for 30 min in basal medium containing or lacking 1.0 mM SITS, the islets were incubated for the indicated periods of time in basal medium supplemented with 0.02 mM ^{3}H-labelled glibenclamide (346 mCi/mmol) as well as either 0.5 mM [^{14}C]urea (20 mCi/mmol) or 0.5 mM 3-*O*-methyl-D-[U-^{14}C]glucose (20 mCi/mmol). In addition the radioactive media contained or lacked SITS, as during preliminary incubation. The islet uptake of glibenclamide in excess of the urea or 3-*O*-methyl-D-glucose spaces is given as mean values ± S.E.M. for the indicated numbers of experiments. The corresponding values for total islet uptake of glibenclamide are stated in parentheses. Effects of SITS were judged from the differences between paired incubations in the presence and absence of SITS.
* $P < 0.02$; ** $P < 0.01$; *** $P < 0.001$

Table 2
Effects of SITS and glibenclamide on insulin release.

Rate of insulin release (ng/μg dry wt./phr)			
Conen. of SITS	0 mM Glibenclamide	0.1 mM Glibenclamide	Effect of glibenclamide
0 mM	0.48 ± 0.07	2.69 ± 0.36	2.21 ± 0.35***
1 mM	3.02 ± 0.55	3.05 ± 0.49	0.03 ± 0.47
Effect of SITS	2.54 ± 0.53***	0.36 ± 0.44	−2.18 ± 0.69** (interaction)

Islets were subjected to preliminary incubation for 40 min in basal medium containing 1 mg of serum albumin/ml as well as 0 or 1.0 mM SITS. They were then incubated for 60 min in fresh media containing the following combinations of 0.1 mM glibenclamide and 1.0 mM SITS: glibenclamide plus SITS, glibenclamide alone, SITS alone, neither glibenclamide nor SITS Islets exposed to SITS were incubated with this compound during the preliminary incubation also. Thirteen different experiments were performed. In each of these, parallel 60-min incubations were carried out with the 4 different media. Amounts of insulin released during the 60-min period are given as mean values ± S.E.M. for each medium. In addition, the effects of glibenclamide and SITS have been calculated from the differences between parallel incubations. ** $P < 0.01$; *** $P < 0.001$

was also present in the glibenclamide-free media. Insulin was assayed radioimmunologically using crystalline mouse insulin as a reference. It was ascertained that SITS did not affect the insulin assay. After the incubations, the islets were freeze-dried, weighed and, in studies of glibenclamide uptake, analyzed for ^{3}H and ^{14}C as previously described [1, 2].

Control experiments were performed to check for strong interactions between SITS and glibenclamide in the absence of islets. Krebs–Ringer bicarbonate medium containing 1 mM SITS and 0.2 mM glibenclamide was incubated at 37°C for 60 min. Samples of medium were then subjected to one dimensional thin-layer chromatography on silica gel (Kieselgel F 254, E. Merck A.G., Darmstadt, W. Germany) using two different solvent systems: 1) chloroform–acetic acid–methanol (95:1:5 by vol.) or 2) butyl acetate–isopropanol–water–25% ammonia in water (30:50:15:5 by vol.). Each sample consisted of 70 μl of medium placed in the starting position as 7 successive 10 μl loads. After chromatography the locations of SITS and glibenclamide were visualized in UV light.

3. Results and discussion

Previous studies have shown that the islet uptake of glibenclamide reaches steady-state after about 45 min [2]. To reveal the effects of SITS on the initial as well as on the steady-state uptake of glibenclamide, the islets were incubated for 3 and 60 min. Table 1 shows that 1 mM SITS significantly inhibited the islet uptake of glibenclamide at both incubation times. After 60 min SITS had depressed the uptake of glibenclamide in excess of the urea space by 40%, suggesting a considerable reduction of the binding to plasma membranes.

Table 2 shows that 1 mM SITS alone stimulates insulin release. This effect of SITS will be described in detail elsewhere [8]. As is also shown in table 2, SITS interacted negatively with the secretagogic action of glibenclamide; the combination of SITS and glibenclamide resulted in a secretory rate that was significantly lower than the sum of rates induced by either SITS or glibenclamide alone ($P < 0.01$). In contradistinction, SITS did not inhibit the insulin-releasing action of glucose [8]. While 10 mM glucose made the islets release insulin at a rate of 3.54 ± 0.63 ng/μg dry wt./hr (mean ± S.E.M. for 8 experiments) the rate recorded after incubation with 10 mM glucose plus 1 mM SITS was 7.39 ± 0.96 ng/μg dry wt./hr (mean ± S.E.M. for 8 experiments).

The results suggest that the insulin-releasing action of glibenclamide depends on the β-cell uptake of this drug as measured by the present technique. Since furthermore SITS is a specific probe of the plasma membrane in other cells [3, 4], it is reasonable to assume that by reacting with the β-cell plasma membrane SITS prevented the secretagogic recognition of glibenclamide. There seems to be no previous evidence for an inhibition of sulfonylurea-induced insulin release at the site of drug recognition.

The alternative explanation that SITS reacted directly with the glibenclamide molecule resulting in a compound with low affinity for β-cells and lacking insulin-releasing capacity seems unlikely in view of the chromatographic control experiments. Both chromatographic procedures resolved the mixture of SITS and glibenclamide into two distinct spots which moved like free SITS and free glibenclamide. In system I SITS remained in the starting position while glibenclamide had a R_f value of 0.28. In system II the R_f of SITS was 0.06 and that of glibenclamide was 0.41. No other spots were observed.

Besides reinforcing the hypothesis that sulfonylureas act on the β-cell plasma membrane, the results reported here indicate that SITS may be useful for elucidating the molecular nature of this action. The probability that SITS reacts with amino [5] and thiol [4] groups in the membranes of other cells draws attention to the possible involvement of such groups in the secretagogic recognition of sulfonylureas. Sulfhydryl reagents, including slowly permeating organic mercurials and iodoacetamide, are known to stimulate insulin release promptly [9, 10]. SITS markedly inhibits the islet uptake and the insulin-releasing capacity of chloromercuribenzene-*p*-sulphonic acid, a strongly secretagogic thiol reagent [11]. Another characteristic property of SITS is its ability to block diffusion into distinct anion channels in the erythrocyte plasma membrane [12]. It seems natural to ask whether the site of action of sulfonylureas is related to a similar channel in the β-cells. Studies are in progress to help answer these questions.

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