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Stimulation and Inhibition of Insulin Release by an Amino-Reactive Probe of Plasma Membrane

Bo Hellman, Lars-Åke Idahl, Åke Lernmark, Janove Sehlin and Inge-Bert Täljedal

Department of Histology, University of Umeå, Umeå, Sweden

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Summary. 4-Acetamido-4′-isothiocyanostilbene-2,2′-disulphonic acid (SITS), an amino-reacting probe of plasma membranes, stimulated the release of insulin from microdissected pancreatic islets of *ob/ob*-mice. This effect of SITS was inhibited by adrenaline or by calcium deficiency. SITS did not inhibit the insulin-releasing action of glucose or leucine but rather potentiated the effect of glucose. In contrast, SITS markedly depressed the insulin secretory response to chloromercuribenzene-*p*-sulphonic acid. It is suggested that by reacting with the plasma membranes SITS may induce secretagogic ionic fluxes in the *β*-cells. In addition, SITS apparently inhibits the secretagogic recognition of chloromercuribenzene-*p*-sulphonic acid, presumably by preventing the organic mercurial from reacting with certain membrane thiol groups.

The amino reactive agent 4-acetamido-4′-isothiocyanostilbene-2,2′-disulphonic acid (SITS) does not penetrate intact erythrocytes or liver cells. It reacts exclusively with the plasma membranes (Maddy, 1964; Marinetti & Gray, 1967; Knauf & Rothstein, 1971*a*) and, in the case of erythrocytes, inhibits the permeation of chloromercuribenzene-*p*-sulphonic acid (CMBS) and small anions (Knauf & Rothstein, 1971*a*, *b*). CMBS and iodoacetamide stimulate insulin release, presumably by reacting with relatively superficial thiol groups in the plasma membranes of pancreatic *β*-cells (Bloom, Hellman, Idahl, Lernmark, Sehlin & Täljedal, 1972; Hellman, Idahl, Lernmark, Sehlin & Täljedal, 1973*a*). These thiol groups might regulate ionic fluxes that are important for stimulus-secretion coupling. Because CMBS and SITS interact in their effects on ionic flux in erythrocytes (Knauf & Rothstein, 1971*b*), we investigated the possibility of whether insulin release is affected by SITS, alone or in combination with CMBS or natural secretagogues.

Materials and Methods

SITS was obtained from British Drug Houses Ltd. CMBS came from Sigma Chemical Co. Other reagents were also commercially available compounds of analytical

grade. Fresh pancreatic islets were microdissected freehand (Hellerström, 1964) from adult *ob/ob*-mice of the Umeå colony. Incubations were performed at 37 °C using Krebs-Ringer's bicarbonate buffer equilibrated with O_2–CO_2 (95:5) as the basal medium. In studies of insulin release the medium also contained 1 to 5 mg/ml serum albumin. All experiments began with preliminary incubation for 30 to 60 min, allowing the islets to equilibrate with the temperature and ingredients of the medium. In studies of insulin release the islets were then either incubated for 60 min in fresh medium and the amount of insulin released measured at the end of incubation ('batch-type incubations'; Lernmark 1971), or subjected to nonrecirculating perifusion in a micro-chamber allowing determinations of the dynamics of insulin release with time (Idahl, 1972). Insulin was assayed radioimmunologically, using crystalline mouse insulin as reference. At the concentrations used, SITS and CMBS did not affect the insulin assay. For measuring the oxidation of D-(U-^{14}C)glucose the islets were incubated for 60 min after which metabolism was stopped with HCl, $^{14}CO_2$ collected in Hyamine, and counting carried out in a liquid scintillation spectrometer (Hellman, Sehlin & Täljedal, 1971). All incubated islets were freeze-dried and weighed as previously described (Bloom *et al.*, 1972; Idahl, 1972; Hellman *et al.*, 1973*a*).

Results

When microperifused islets were exposed to 1 mM SITS they responded with a marked enhancement of insulin release (Fig. 1). The onset of stimulation occurred about 6 min after the islets were exposed to SITS. After this lag the rate of insulin release rose quickly. Peak values were recorded about 25 min after the onset. In spite of continued perifusion with SITS the release rate then started to decline, although stimulation was evident for another 45 min after which period the experiments were interrupted. Fig. 1 also shows that the stimulatory effect of SITS was markedly depressed when calcium was omitted from, or adrenaline was added to, the perifusion medium. Tables 1 and 2 summarize the results of batch-type incubations which verify statistically that SITS stimulates insulin release and that this effect is inhibited by calcium deficiency or adrenaline. In the presence of 10 mM glucose significant stimulation of insulin release was obtained with both 0.01 and 1.0 mM SITS (Table 2).

Fig. 1 as well as Tables 1 and 2 show that SITS did not inhibit the insulin-releasing action of glucose or leucine; SITS rather potentiated the effect of glucose. In contrast, SITS markedly suppressed the insulin secretory response to CMBS (Fig. 2). As shown in Table 3, SITS did not stimulate but at the concentration of 1 mM probably inhibited glucose oxidation.

Discussion

Insulin release is associated with altered ionic fluxes across the β-cell plasma membrane (Dean & Matthews, 1970*a*, *b*; Pace & Price, 1972). The

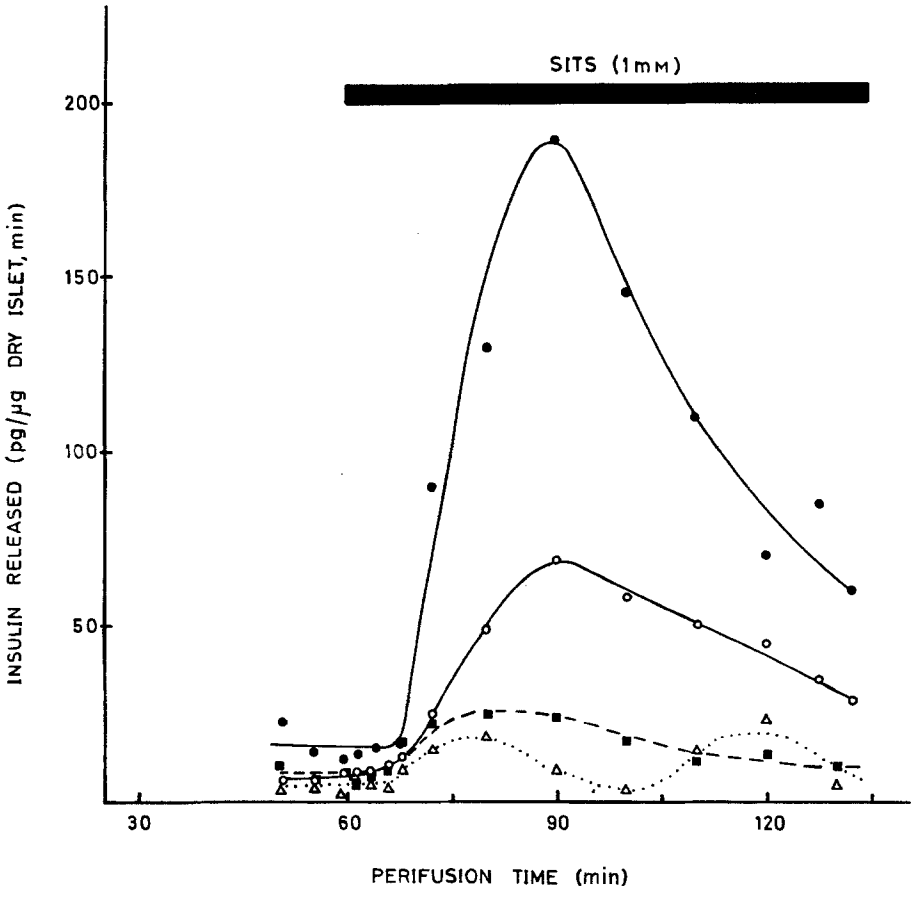

Fig. 1. Dynamics of SITS-induced insulin release. Islets were perifused with medium containing 3 (○) or 17 (●) mM glucose. After 60 min the perifusion medium was suddenly switched to the same kind of medium supplemented with 1 mM SITS. Perifusion with SITS lasted for 75 min (bar). In other experiments calcium was omitted from (■) or 2 µg/ml adrenaline was added to (△) the perifusion medium both before and after the introduction of SITS. In these experiments the glucose concentration was 3 mM. The points represent the average rate of insulin release over each sampling period

nature of these fluxes is not fully understood but they are probably essential for secretion. The understanding of ionic fluxes in erythrocytes has been aided by studying the effects of SITS (Rothstein, 1970; Knauf & Rothstein, 1971*b*), which was originally designed to react specifically with the outer components of plasma membrane (Maddy, 1964). The finding that SITS is an insulin secretagogue suggests that it produces significant changes of ionic flux in the β-cells and may be useful for probing the plasma membrane

Table 1. Effects of SITS and other compounds on insulin release

Composition of medium	No. of expts.	ng Insulin released/μg dry islet per hr			*P*-value for effect of SITS
		0 mM SITS	1 mM SITS	Effect of SITS	
Control	13	0.52±0.07	2.60±0.63	2.08±0.60	<0.01
D-Glucose, 20 mM	12	6.71±1.26	11.39±1.77	4.68±1.44	<0.01
L-Leucine, 20 mM	10	3.88±0.74	8.17±1.81	4.29±1.51	<0.02
Control	6	0.35±0.10	1.51±0.42	1.16±0.39	<0.05
Adrenaline, 0.2 μg/ml	6	0.48±0.20	0.53±0.15[a]	0.05±0.25	>0.05
No Ca^{2+}	6	0.38±0.08	0.63±0.12[b]	0.25±0.06	<0.01

After preliminary incubation for 40 min in glucose-free medium, islets were incubated for 60 min in media containing test substances as indicated. When SITS was used in the 60-min incubation, this compound was also present during the preliminary incubation period. The effect of no Ca^{2+} was tested by excluding $CaCl_2$ from the basal medium during both the preliminary incubation and the subsequent 60-min period. In each experiment parallel incubations were performed with and without SITS. Results are presented as mean values ±SEM for the stated numbers of experiments. Statistical significances were judged from the mean values ±SEM for differences between parallel incubations.

[a] Effect of adrenaline on SITS-induced insulin release: −0.98±0.32 ($P<0.05$).

[b] Effect of no Ca^{2+} on SITS-induced insulin release: −0.88±0.34 ($P<0.05$).

Table 2. Effects of different concentrations of SITS on insulin release

Conc of glucose (mM)	Conc of SITS (mM)	Insulin released (ng/μg dry wt per hr)
0	0	0.56±0.12
0	1.0	2.04±0.83
10	0	3.54±0.63
10	0.01	4.59±0.57[a]
10	0.1	4.92±0.75
10	1.0	7.39±0.96[b]

After preliminary incubation for 40 min in basal medium containing 5 mg/ml serum albumin, islets were incubated for 60 min in the same type of medium supplemented with test substances as indicated. Eight experiments were performed. In each of these, parallel incubations were carried out in all of the six listed media. Results are presented as the mean values ±SEM. Statistical significances were judged from the mean values ±SEM for differences between parallel incubations. Effect of 10 mM glucose without SITS: 2.98±0.64 ($P<0.005$). Effect of 10 mM glucose in the presence of 1 mM SITS: 5.36±0.68 ($P<0.001$). Potentiation of 10 mM glucose by 1 mM SITS: 2.37±0.95 ($P<0.05$). Effects of SITS versus 10 mM glucose alone: [a] $P<0.02$; [b] $P<0.005$.

of these cells. Since glucose might stimulate insulin release by being metabolized in the β-cells (Ashcroft, Bassett & Randle, 1972), it is notable that SITS did not stimulate but at the concentration of 1 mM probably inhibited

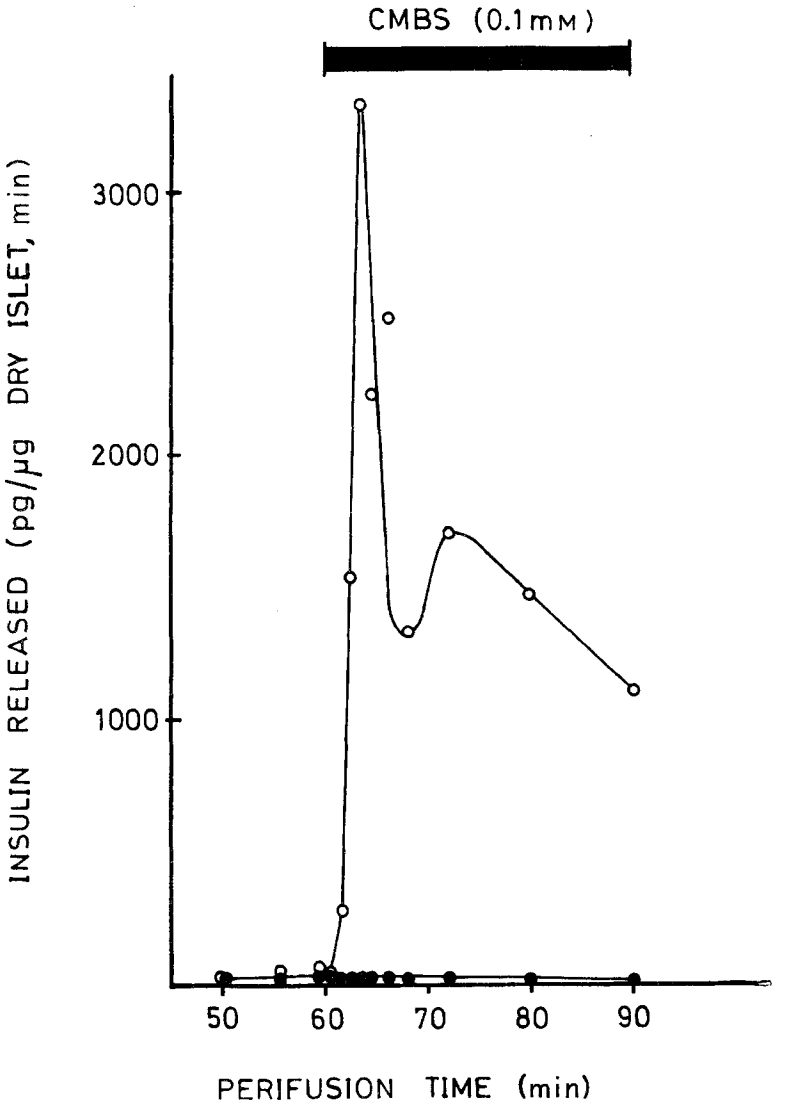

Fig. 2. Effect of SITS on insulin release in response to chloromercuribenzene-*p*-sulphonic acid (CMBS). Islets were perifused with medium containing 3 mM glucose. After 60 min the medium was suddenly supplemented with 0.1 mM CMBS. Perifusion with CMBS is indicated by the bar. In one type of experiment (•) 1 mM SITS was also present from 10 min before the introduction of CMBS until the experiment was interrupted. Control experiments (○) were performed without SITS. The points represent the average rate of insulin release over each sampling period. (The insulin-releasing action of CMBS has been characterized in detail elsewhere; Bloom *et al.*, 1972)

glucose oxidation. This tendency to inhibit glucose oxidation does not necessarily mean that the β-cells differ from erythrocytes by being permeable to SITS. The glucose metabolism in pancreatic islets can be inhibited by

Table 3. Effect of SITS on glucose oxidation

Conc of SITS (mM)	Rate of glucose oxidation
Nil (control)	25.5 ± 2.31
0.01	25.8 ± 2.55
1.0	19.0 ± 2.86[a]

After preliminary incubation in basal medium containing 3 mM glucose, islets were incubated for 60 min in basal medium supplemented with 10 mM (U-^{14}C)-D-glucose (1.7 mC/mmole) as well as with SITS as indicated. Amounts of $^{14}CO_2$ liberated are expressed as mmole glucose equivalents completely oxidized/kg dry weight of islets per hour. Mean values ± SEM are given for 6 different experiments.

[a] $P<0.05$, as judged from the mean value ± SEM of differences versus paired control incubations.

manipulating the ionic composition of medium (Ashcroft *et al.*, 1972), and SITS may affect the flux of ions in the β-cells.

The potential value of SITS as a probe of the β-cell plasma membrane is perhaps even more striking in view of the interactions that were observed between SITS and other stimuli of insulin release. In contrast to the synergism between SITS and glucose as insulin secretagogues, SITS markedly suppressed the secretory response to CMBS. Hence, SITS probably interferes with the secretagogic recognition of this sulfhydryl reagent but does not inhibit the mechanisms for insulin discharge. From the point of view of chemical structure, a direct interaction between the SITS and CMBS molecules would not be expected. It was recently found that SITS inhibits both the islet uptake and the insulin-releasing action of glibenclamide, a widely-used hypoglycemic sulfonylurea derivative (Hellman, Lernmark, Sehlin & Täljedal, 1973*b*). This resemblance between a typical insulin-releasing sulfonylurea and a potently secretagogic thiol reagent suggests that the recognition of these two classes of secretagogues may have some significant features in common.

We have previously suggested that CMBS stimulates insulin release by reacting with relatively superficial thiol groups in the β-cell plasma membrane (Bloom *et al.*, 1972). Whether these thiol groups are located on or below the surface of the β-cells was not specified. In erythrocytes SITS has been used to distinguish between thiol groups at different depths of the membrane. Whereas SITS has little effect on the most superficial binding of CMBS, it strongly inhibits the diffusion of CMBS into the membrane (Rothstein, 1970; Knauf & Rothstein, 1971*b*). Assuming that SITS has a similar effect on the β-cells, its inhibition of insulin release in response to

CMBS suggests that the thiol groups involved in the secretagogic recognition of sulfhydryl reagents are located below the surface of the β-cells. This conclusion encourages the viewpoint that the effect of individual sulfhydryl reagents on insulin release may depend not only on their chemical reactivity but also on their ability to penetrate the plasma membrane and reach their target thiol groups (Bloom *et al.*, 1972; Hellman *et al.*, 1973*a*). Further studies are needed to clarify the question of whether SITS has the assumed effect on the permeation of CMBS in β-cell membranes.

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References

Ashcroft, S. J. H., Bassett, J. M., Randle, P. J. 1972. Insulin secretion mechanisms and glucose metabolism in isolated islets. *Diabetes* **21** (suppl. 2):538

Bloom, G. D., Hellman, B., Idahl, L.-Å., Lernmark, Å., Sehlin, J., Täljedal, I.-B. 1972. Effects of organic mercurials on mammalian pancreatic β-cells. Insulin release, glucose transport, glucose oxidation, membrane permeability and ultrastructure. *Biochem. J.* **129**:241

Dean, P. M., Matthews, E. K. 1970*a*. Glucose-induced electrical activity in pancreatic islets cells. *J. Physiol.* **210**:255

Dean, P. M., Matthews, E.K. 1970*b*. Electrical activity in islet cells: Effect of ions. *J. Physiol.* **210**:265

Hellerström, C. 1964. A method for the microdissection of intact pancreatic islets of mammals. *Acta Endocr., Copenhagen* **45**:122

Hellman, B., Idahl, L.-Å., Lernmark, Å., Sehlin, J., Täljedal, I.-B. 1973*a*. Iodoacetamide-induced sensitization of the pancreatic β-cells to glucose stimulation. *Biochem. J.* **132**:775

Hellman, B., Lernmark, Å., Sehlin, J., Täljedal, I.-B. 1973*b*. The pancreatic β-cell recognition of insulin secretagogues. VI. Inhibitory effects of a membrane probe on the islet uptake and insulin-releasing action of glibenclamide. *F.E.B.S.* **34**:347

Hellman, B., Sehlin, J., Täljedal, I.-B. 1971. Effects of glucose and other modifiers of insulin secretion on the oxidative metabolism of amino acids in microdissected pancreatic islets. *Biochem. J.* **123**:513

Idahl, L.-Å. 1972. A micro perifusion device for pancreatic islets allowing concomitant recordings of intermediate metabolites and insulin release. *Analyt. Biochem.* **50**:386

Knauf, P. A., Rothstein, A. 1971*a*. Chemical modifications of membranes. I. Effects of sulfhydryl and amino reactive reagents on anion and cation permeability of the human red blood cell. *J. Gen. Physiol.* **58**:190

Knauf, P. A., Rothstein, A. 1971*b*. Chemical modifications of membranes. II. Permeation paths for sulfhydryl agents. *J. Gen. Physiol.* **58**:211

Lernmark, Å. 1971. Isolated mouse islets as a model for studying insulin release. *Acta Diabet. Lat.* **8**:649

Maddy, A. H. 1964. A fluorescent label for the outer components of the plasma membrane. *Biochim. Biophys. Acta* **88**:390

Marinetti, G. V., Gray, G. M. 1967. A fluorescent chemical marker for the liver cell plasma membrane. *Biochim. Biophys. Acta* **135**:580

Pace, C. S., Price, S. 1972. Electrical responses of pancreatic islet cells to secretory stimuli. *Biochem. Biophys. Res. Commun.* **46**:1557

Rothstein, A. 1970. Sulfhydryl groups in membrane structure and function. *Curr. Top. Membranes Transp.* **1**:135