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Role of Thiol Groups in Insulin Release: Studies with Poorly Permeating Disulphides

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SUMMARY

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At a concentration of 1.0 mM, 6,6′-dithiodinicotinic acid and 5,5′-dithiobis(2-nitrobenzoic acid) stimulated insulin release from microdissected pancreatic islets of hereditary obese (*ob/ob*) mice. Microperifusion experiments showed that the secretory responses occurred promptly upon exposure to the sulphydryl reagents. Perifusion with 6,6′-dithiodinicotinic acid induced a sustained enhancement of insulin release without any signs of multiphasic secretion. This reagent induced a similar release pattern at both 3 and 17 mM glucose, although the high glucose concentration appeared to potentiate the effect of 6,6′-dithiodinicotinic acid. The dynamics of insulin release in response to 5,5′-dithiobis(2-nitrobenzoic acid) depended markedly, however, on the accompanying glucose concentration. At 0 and 3 mM glucose an initial peak of release was followed by a steady decline towards the basal release rate, whereas at 17 mM glucose 5,5′-dithiobis(2-nitrobenzoic acid) produced a sustained enhancement of secretion. The omission of calcium significantly inhibited insulin release in response to either of the two disulphides, although clear-cut stimulation was still obtained. In marked contrast, 17 mM glucose did not elicit even an initial peak of insulin release when calcium was omitted from the perifusion medium. 6,6′-Dithiodinicotinic acid (0.01–1.0 mM) had no effect on the oxidation of [U-^{14}C]D-glucose. Significant inhibition of glucose oxidation was obtained with 0.1 and 1.0 mM 5,5′-dithiobis(2-nitrobenzoic acid), whereas at a concentration of 0.01 mM this reagent stimulated oxidation. Since the disulphides do not readily penetrate cell membranes, the results are consistent with our hypothesis that insulin release is regulated by relatively superficial thiol groups in the β-cell plasma membrane. However, further studies are necessary to exclude the possibility that the observed effects were due to small amounts of disulphide entering the β-cells.

INTRODUCTION

Two different types of sulphydryl reagents are known to stimulate insulin release: chloromercuribenzene-*p*-sulphonic acid and *p*-chloromercuribenzoic acid (1), which form readily reversible mercaptides, and iodoacetamide (2), a more irreversibly reacting alkylating compound. The organic mercurials stimulated insulin release equally well at 3 mM and 17 mM glucose (1), whereas the effect of iodoacetamide was markedly

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glucose dependent (2). Since chloromercuribenzene-*p*-sulphonic acid penetrates cell membranes slowly, it was suggested that insulin release may be regulated by relatively superficial thiol groups in the β-cell plasma membrane. More precisely, the hypothesis was put forward that the reaction of sulphydryl reagents with these thiol groups causes the activation of some component of the physiological release mechanism (1, 2).

To test this hypothesis further, we have extended our studies to include a third class of sulphydryl reagents. 5,5′-Dithiobis-(2-nitrobenzoic acid) and 6,6′-dithiodinicotinic acid react with thiol groups in tissues to form disulphide bridges between tissue and reagent or between neighbouring thiol groups in the tissue (3–5). Both compounds are electronegative disulphides with a poor capacity to enter cells (4). It will be shown that both 5,5′-dithiobis(2-nitrobenzoic acid) and 6,6′-dithiodinicotinic acid stimulate insulin release but differ with respect to their dependence on glucose concentration. The mechanism of insulin release in response to sulphydryl reagents will be discussed in relation to the differences in glucose dependence and the inhibitory effects of calcium deficiency.

MATERIALS AND METHODS

6,6′-Dithiodinicotinic acid (lot C 671) was obtained from Newcell Biochemicals, Berkeley, Cal., and 5,5′-dithiobis(2-nitrobenzoic acid) from British Drug Houses, Ltd. ^{125}I-Labelled insulin was purchased from Novo A/S, Copenhagen, and from Farbwerke Hoechst A. G., Frankfurt/Main, and insulin antibodies from Novo A/S and Wellcome Reagents, Ltd., Beckenham, U.K. [U-^{14}C]D-Glucose was obtained from The Radiochemical Rentre, Amersham, U. K. All other compounds were commercially available reagents of analytical grade. Distilled, deionized water was used throughout.

Islets were microdissected freehand (6) from the pancreatic glands of adult *ob/ob* mice which had been fasted overnight. The animals were taken from a local colony that has been bred in our laboratory for more than 10 years. Krebs-Ringer-bicarbonate buffer equilibrated with O_2–CO_2 (95:5) was used as the basal medium in all incubations. In studies of insulin release, the medium also contained 1 mg of albumin per milliliter, whereas albumin-free media were used in the glucose oxidation experiments. Further additions to the media are described in the legends to figures and tables.

Two different methods were used for measuring insulin release, both of which have been described in detail elsewhere (1, 7, 8). In one type of experiment, batches of two islets were incubated for 60 min in 300 μl of medium, and insulin was determined in samples of medium taken at the end of the incubation period. This technique, which measures the mean rate of insulin release over 1 hr, will be referred to as "batch type" incubations. The dynamics of insulin release was studied with the aid of a microperifusion apparatus, which permits continuous sampling of insulin at various intervals during incubation (8). Two islets were placed in a polythene-nylon chamber with a volume of 4.5 μl and were perifused at a rate of 33–35 μl/min. Two reservoirs were connected with the perifusion chamber in such a way as to allow sudden alterations of the medium fed to the islets. In each perifusion experiment three different chambers were loaded with islets from a single animal and run in parallel. Results given are mean values for these three chambers. The representative nature of published curves was assessed in repeated experiments. Insulin was assayed radioimmunologically, using ethanol to separate free and antibody-bound insulin (9). At the concentrations used, the disulphides did not affect the insulin assay. Glucose oxidation was measured by incubating single islets as previously described (10) in 100 μl of medium containing 10 mM [U-^{14}C]D-glucose (1.7 mCi/mmole). After the incubations, all islets were freeze-dried and weighed on quartz fibre balances (1); rates of insulin release and glucose oxidation were expressed per unit of dry weight of islets. Unless otherwise stated, statistical testing was carried out by com-

puting t values from the mean ± standard error of differences between paired observations.

RESULTS

Table 1 shows the effects of different concentrations of 6,6′-dithiodinicotinic acid or 5,5′-dithiobis(2-nitrobenzoic acid) on the release of insulin in batch type incubations for 60 min. Although both substances seemed to elicit a dose-dependent release from 0.01 to 1.0 mM, stimulation was significant only with 1.0 mM 6,6′-dithiodinicotinic acid and with 0.1 or 1.0 mM 5,5′-dithiobis(2-nitrobenzoic acid). Stimulation was obtained whether the accompanying glucose concentration was 3 mM or 10 mM. None of the sulphydryl reagents seemed to inhibit glucose-stimulated insulin release.

The dynamics of insulin release in response to 1.0 mM 6,6′-dithiodinicotinic acid or 1.0 mM 5,5′-dithiobis(2-nitrobenzoic acid) is shown in Figs. 1–5. 6,6′-Dithiodinicotinic acid rapidly stimulated insulin release at both 3 mM and 17 mM glucose (Fig. 1). Stimulation was evident during the whole perifusion period (75 min), there being no signs of a multiphasic release pattern. The corresponding records of insulin release in response to 5,5′-dithiobis-(2-nitrobenzoic acid) are shown in Fig. 2. When tested in a glucose-free medium, 5,5′-dithiobis(2-nitrobenzoic acid) caused a moderate initial peak of insulin release, which was soon followed by a return to the basal rate. In the presence of 3 mM glucose, 5,5′-dithiobis(2-nitrobenzoic acid) quickly raised the release rate to a level as high as about 250 pg of insulin per microgram of islets, dry weight, per minute. After a few minutes the release rate started to fall, resulting in a distinct initial peak. A qualitatively different pattern was obtained when 5,5′-dithiobis(2-nitrobenzoic acid) was tested in combination with 17 mM glucose. Again 5,5′-dithiobis(2-nitrobenzoic acid) caused a prompt stimulation of insulin release. No initial peak was observed, but insulin release steadily approached a rate well above that recorded with 17 mM glucose alone.

When islets were exposed to a brief pulse of 1.0 mM 6,6′-dithiodinicotinic acid, the rate of insulin release started to rise but fell again upon withdrawal of the reagent (Fig. 3). In contrast, in the presence of 17 mM

TABLE 1

Effects of different concentrations of 6,6′-dithiodinicotinic acid and 5,5′-dithiobis(2-nitrobenzoic acid) on insulin release

After preliminary incubation with 3 mM glucose for 40 min, the islets were incubated for 60 min with the disulphide and glucose as indicated. Amounts of insulin released during the final 60 min are presented as mean values ± standard errors for the numbers of experiments shown in parentheses.

Disulphide	Concentration	Insulin released	
		3 mM glucose	10 mM glucose
	mM	*ng/hr/μg islets (dry wt)*	
6,6′-Dithiodinicotinic acid	0[a]	1.30 ± 0.21 (8)	4.61 ± 1.12 (7)
	0.01	1.79 ± 0.49 (7)	5.38 ± 1.09 (8)
	0.1	2.05 ± 0.50 (8)	5.26 ± 1.04 (8)
	1.0	4.01 ± 0.54[b] (8)	6.65 ± 0.76[c] (7)
5,5′-Dithiobis(2-nitrobenzoic acid)	0[a]	0.57 ± 0.15 (8)	4.83 ± 1.57 (8)
	0.01	1.13 ± 0.43 (7)	5.07 ± 1.49 (8)
	0.1	2.35 ± 0.55[d] (8)	7.46 ± 1.85[c] (8)
	1.0	2.80 ± 0.40[d] (8)	7.19 ± 1.47[c] (8)

[a] Control.
[b] $p < 0.001$ compared with control.
[c] $p < 0.05$ compared with control.
[d] $p < 0.01$ compared with control.

glucose, the effect of 1.0 mM 5,5′-dithiobis-(2-nitrobenzoic acid) was not readily reversible (Fig. 3). After 8 min of perifusion with 5,5′-dithiobis(2-nitrobenzoic acid) the islets released insulin at a stimulated rate that was maintained for almost 1 hr.

In addition to showing that 6,6′-dithiodinicotinic acid stimulates insulin release at both 3 mM and 17 mM glucose, Fig. 1 suggests that the effect of 6,6′-dithiodinicotinic acid is more pronounced at the high glucose concentration. Similarly, in a

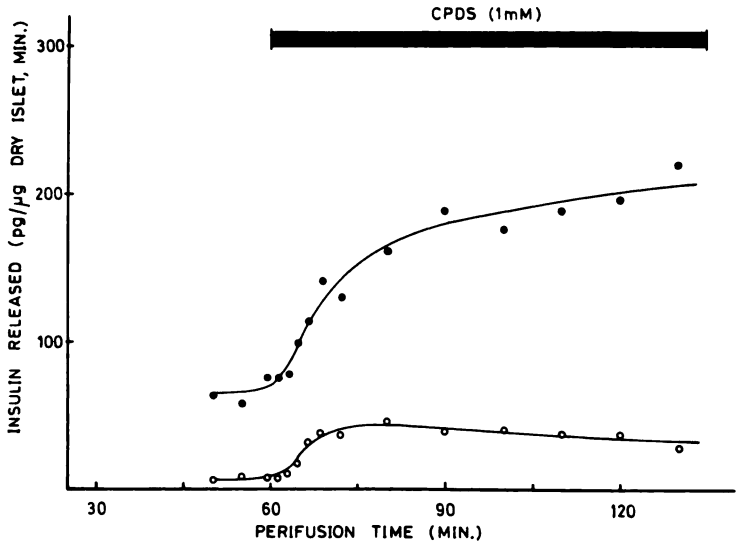

FIG. 1. *Dynamics of insulin release in response to 6,6′-dithiodinicotinic acid (CPDS)*
Islets were perifused with 3 mM (○) or 17 mM (●) glucose. After 60 min 1 mM 6,6′-dithiodinicotinic acid was added to the medium, and perifusion with disulphide was continued for 75 min (bar). The points represent the average rate of insulin release over each sampling period.

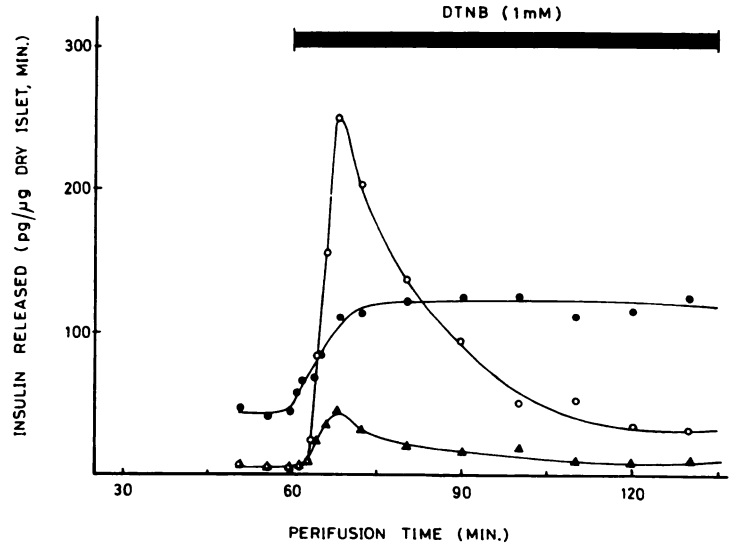

FIG. 2. *Dynamics of insulin release in response to 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB)*
The experiments were designed as in Fig. 1, with the exception that three different concentrations of glucose were used: 0 mM (▲), 3 mM (○), and 17 mM (●).

series of batch type incubations with 1 mM 6,6′-dithiodinicotinic acid, the disulphide-induced secretory response seemed to increase with the glucose concentration (Table 2). However, as judged from an analysis of variance, the apparent potentiation between glucose and 6,6′-dithiodinicotinic acid was not statistically significant. Such a study was not performed with 5,5′-dithiobis(2-nitrobenzoic acid), since the qualitatively different patterns of release at different glucose concentrations (Fig. 2) precluded a meaningful analysis of the interaction with glucose in batch type incubations.

As shown in Figs. 4 and 5, an initial peak of insulin release was recorded when islets were perifused with 5,5′-dithiobis(2-nitro-

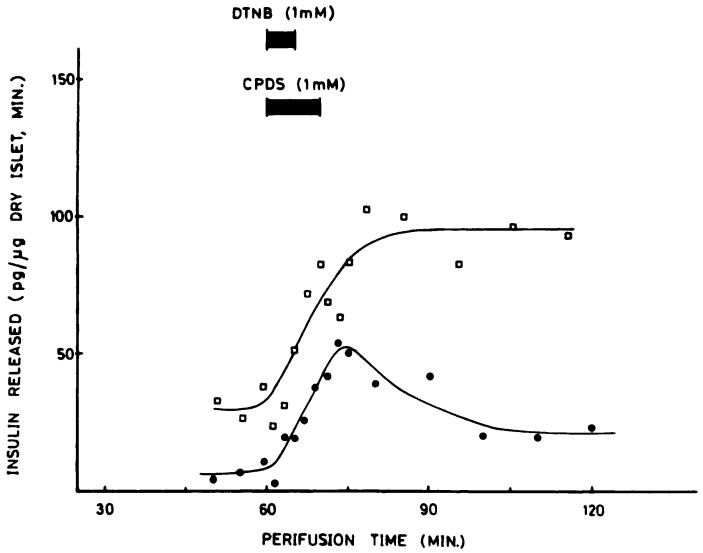

FIG. 3. *Dynamics of insulin release in response to brief pulse of 1 mM 6,6′-dithiodinicotinic acid (CPDS, ●) or 1 mM 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB, □)*

The disulphides were included in the medium as indicated by the solid bars. The glucose concentration was 3 mM throughout the experiment with 6,6′-dithiodinicotinic acid and 17 mM throughout that with 5,5′-dithiobis(2-nitrobenzoic acid). The points represent the average rate of insulin release over each sampling period.

TABLE 2

Effects of 6,6′-dithiodinicotinic acid on insulin release in the presence of different concentrations of glucose

Islets were first incubated for 60 min with the listed concentrations of glucose. They were then incubated for another period of 60 min in medium containing the same concentrations of glucose as well as 0 or 1 mM 6,6′-dithiodinicotinic acid. Amounts of insulin released during the final 60 min are presented as in Table 1.

Glucose	Insulin released		
	No 6,6′-dithiodinicotinic acid	1 mM 6,6′-dithiodinicotinic acid	Effect of 6,6′-dithiodinicotinic acid
mM		*ng/hr/µg islets (dry wt)*	
0	0.45 ± 0.11 (8)	1.14 ± 0.20 (8)	0.69 ± 0.20[a] (8)
5	0.71 ± 0.15 (8)	1.80 ± 0.50 (8)	1.09 ± 0.57 (8)
10	2.85 ± 0.61 (8)	4.17 ± 0.72 (8)	1.32 ± 1.03 (8)
20	5.59 ± 0.60 (8)	8.70 ± 0.60 (8)	3.11 ± 1.01[a] (8)

[a] $p < 0.02$.

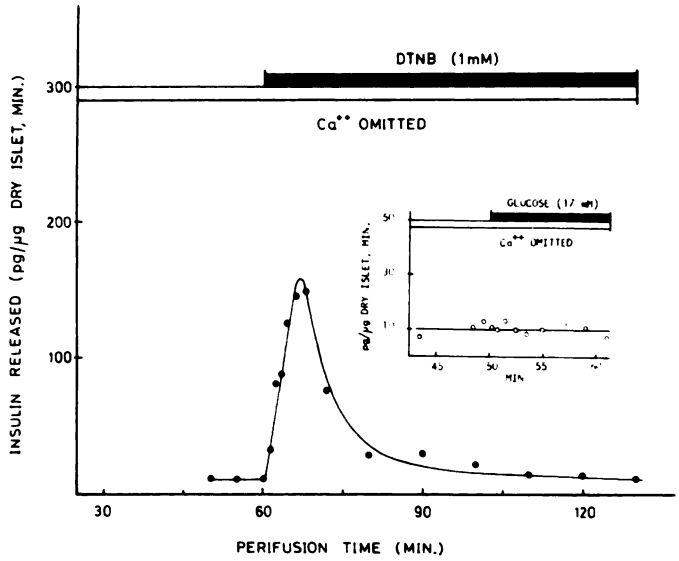

FIG. 4. *Effect of calcium deficiency on dynamics of insulin release in response to 5,5'-dithiobis(2-nitrobenzoic acid (DTNB) or glucose*

In all media $CaCl_2$ was replaced by equimolar NaCl. The main diagram shows the effect of 1 mM 5,5'-dithiobis(2-nitrobenzoic acid) (solid bar) in the presence of 3 mM glucose. The inset shows a corresponding experiment in which the islets were first exposed to 3 mM glucose and after 50 min to 17 mM glucose. In the latter experiment no disulphide was used. The points represent the average rate of insulin release over each sampling period.

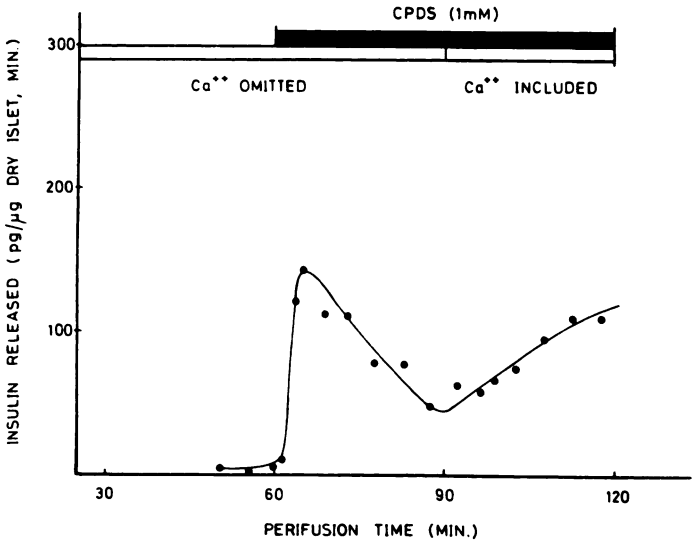

FIG. 5. *Effect of calcium deficiency on dynamics of insulin release in response to 6,6'-dithiodinicotinic acid (CPDS)*

The islets were perifused for 90 min with a medium in which $CaCl_2$ had been replaced by equimolar NaCl. During the last 30 min of this period 1 mM 6,6'-dithiodinicotinic acid was also present in the medium, and perifusion with the disulphide was continued for 60 min (solid bar). After 30 min of exposure to the disulphide, 5 mEq of Ca^{2+} were added. The glucose concentration was 17 mM throughout. This glucose concentration did not stimulate insulin release when calcium was omitted. The points represent the average rate of insulin release over each sampling period.

benzoic acid) or 6,6′-dithiodinicotinic acid in a calcium-free medium. However, Fig. 5 also shows that calcium deficiency inhibited insulin release in response to 6,6′-dithiodinicotinic acid. The release rate was not maintained at the initial high level (cf. Fig. 1), but declined until calcium was again introduced into the perifusion medium. The batch type incubation data of Table 3 suggest that the stimulatory effect of 5,5′-

TABLE 3

Effects of calcium deficiency on insulin release in response to glucose, 6,6′-dithiodinicotinic acid, and 5,5′-dithiobis(2-nitrobenzoic acid)

Islets were first incubated for 40 min in medium containing 3 mM glucose and 0 or 5 mEq of Ca^{2+}. They were then incubated for 60 min with the same concentrations of calcium, as well as glucose and disulphide as indicated. Amounts of insulin released during the final 60 min are presented as in Table 1. $CaCl_2$ was replaced by equimolar concentration of NaCl in calcium-deficient media.

Disulphide	Disulphide concentration	Glucose concentration	Insulin released	
			5 mEq Ca^{2+}	No Ca^{2+}
	mM	*mM*	*ng/hr/μg islets (dry wt)*	
6,6′-Dithiodinicotinic acid	0	3	1.51 ± 0.45 (8)	2.16 ± 0.54 (8)
	1	3	3.01 ± 0.39 (8)	3.21 ± 0.62 (7)
	0	10	5.72 ± 0.79 (8)	2.91 ± 0.52[a] (8)
	1	10	9.69 ± 1.71 (8)	1.49 ± 0.28[a] (8)
5,5′-Dithiobis(2-nitrobenzoic acid)	0	3	1.95 ± 0.53 (7)	1.43 ± 0.50 (7)
	1	3	3.70 ± 0.64 (7)	1.85 ± 0.18[b] (7)
	0	10	5.44 ± 1.06 (7)	1.25 ± 0.37[c] (7)
	1	10	7.69 ± 1.67 (7)	3.54 ± 0.92[c] (7)

[a] $p < 0.01$ compared with corresponding value at 5 mEq of Ca^{2+}.
[b] $p < 0.05$ compared with corresponding value at 5 mEq of Ca^{2+}.
[c] $p < 0.02$ compared with corresponding value at 5 mEq of Ca^{2+}.

TABLE 4

Effects of 6,6′-dithiodinicotinic acid and 5,5′-dithiobis(2-nitrobenzoic acid) on glucose oxidation

Islets were first incubated for 45 min in medium containing 3 mM glucose. They were then incubated for 60 min with 10 mM [U-^{14}C]D-glucose (1.7 mCi/mmole) and the indicated concentrations of the disulphide. Parallel control incubations were performed without the disulphide in the medium. Amounts of $^{14}CO_2$ liberated are expressed in terms of glucose equivalents oxidized. Results are given as mean values ± standard errors for each test medium as well as for the differences between parallel test and control incubations. The numbers of experiments are shown in parentheses.

Disulphide	Concentration	Rate of oxidation	
		Test value	Test minus control
	mM	*mmoles/hr/kg islets (dry wt)*	
6,6′-Dithiodinicotinic acid	0.01	26.3 ± 4.6 (6)	−7.2 ± 5.2 (6)
	0.1	38.7 ± 7.0 (6)	5.2 ± 7.6 (6)
	1.0	32.4 ± 4.7 (6)	3.7 ± 3.9 (6)
5,5′-Dithiobis(2-nitrobenzoic acid)	0.01	37.1 ± 5.0 (5)	11.9 ± 4.0[a] (5)
	0.1	11.9 ± 6.7 (4)	−18.1 ± 4.4[a] (4)
	1.0	16.6 ± 3.3 (5)	−8.6 ± 2.3[b] (5)

[a] $p < 0.05$.
[b] $p < 0.025$.

dithiobis(2-nitrobenzoic acid) was probably also decreased by calcium deficiency, at least at a low glucose concentration. It must be stressed that omitting calcium from the incubation medium probably does not result in a total disappearance of calcium from the incubated or perifused islets. Therefore the dynamic records in Figs. 4 and 5 do not rule out the possibility that calcium ion is important for the initial secretory responses to 6,6′-dithiodinicotinic acid and 5,5′-dithiobis(2-nitrobenzoic acid). They indicate, however, that these sulphydryl reagents have a requirement for calcium that is different from that of glucose. When calcium was omitted from the perifusion medium, there was not even a tendency to stimulation in response to 17 mM glucose (Fig. 4). In contrast, a clear-cut initial response to 5,5′-dithiobis(2-nitrobenzoic acid) was still observed when ethylene glycol bis(β-aminoethyl ether)-N,N′-tetraacetic acid was included as a calcium-chelating agent in the medium (not shown).

Table 4 shows that 0.01–1.0 mM 6,6′-dithiodinicotinic acid had no significant effect on the oxidation of glucose by microdissected islets. Significant inhibition was observed with 0.1 and 1.0 mM 5,5′-dithiobis(2-nitrobenzoic acid), whereas at a concentration of 0.01 mM this reagent stimulated the oxidation of glucose (Table 4).

DISCUSSION

Douglas *et al.* (11) and Schofield (12) reported that sulphydryl reagents, including 5,5′-dithiobis(2-nitrobenzoic acid), stimulate the release of pituitary hormones. We observed that insulin release is strongly stimulated by organic mercurials at concentrations that had no detectable effect on the β-cell permeability to sucrose or mannitol (1). Since the slowly permeating chloromercuribenzene-*p*-sulphonic acid elicited a prompt secretory response from microperifused islets, it was suggested that insulin release is regulated by relatively superficial thiol groups in the β-cell plasma membrane (1). Sulphydryl reagents were thought to activate some component of the physiological release mechanism through their reaction with these thiol groups. These hypotheses have already passed one test, as it was shown that, contrary to general belief, iodoacetamide is not a typical inhibitor of insulin release but under certain conditions is a powerful insulin secretagogue (2). The results reported here are also consistent with the presence of regulatory thiol groups in the β-cell plasma membrane. Both 5,5′-dithiobis(2-nitrobenzoic acid) and 6,6′-dithiodinicotinic acid caused a prompt release of insulin in the microperifusion system. Although we do not know whether these sulphydryl reagents are capable of entering the β-cells, other studies indicate that they penetrate cell membranes slowly (4).

There is a close correlation between glucose-induced insulin release and glucose metabolism in the pancreatic islets (13). However, insulin release in response to sulphydryl reagents does not seem to be mediated by stimulation of glucose metabolism. Both organic mercurials (1) and iodoacetamide (2) stimulated insulin release strongly at concentrations which had no effect on glucose oxidation. In the present study 1.0 mM 5,5′-dithiobis(2-nitrobenzoic acid) inhibited glucose oxidation whereas 6,6′-dithiodinicotinic acid had no effect.

It has been suggested that insulin secretagogues directly or indirectly change the conformation of β-cell plasma membrane, resulting in altered ionic fluxes, which in turn activate the mechanism for insulin discharge (14). Blockade of thiol groups with sulphydryl reagents could perhaps result in changes of membrane structure that alter the ion permeability without rendering the β-cells grossly leaky (see refs. 15 and 16). If, as envisaged by Schwartz *et al.* (16), the conformation of plasma membrane is controlled by disulphide bridges, ion permeability might depend on the oxidation-reduction equilibrium between disulphide bridge and thiol groups. In such a model sulphydryl reagents could perhaps stimulate insulin release by shifting the equilibrium away from the disulphide bridge conformation. Glucose-induced insulin release is associated with a striking increase in the β-cells of reduced pyridine nucleotides (17), which might provide the reductive potential that is necessary for a

thiol-disulphide bridge model to work as a physiological mechanism.

The above viewpoints should not obscure the possibility that sulphydryl reagents might affect the β-cells in such a complex way that it cannot be fully explained by any single type of interaction. In particular it seems necessary to consider whether all the investigated reagents are likely to act on the same thiol groups (18, 19). The inability of iodoacetamide to stimulate insulin release in the absence of glucose (2) suggests that there is at least one class of thiol groups blockade of which is not sufficient to produce the secretagogic conformation of the plasma membrane although the β-cells become hyper-responsive to glucose. On the other hand, the glucose independence of organic mercurials (1) and 6,6′-dithiodinicotinic acid suggests that a fully secretagogic conformation can be produced through the blockade of certain other thiols.

Relatively little is known about the nature of ionic fluxes across the β-cell plasma membrane. It has been proposed that recognition of a physiological stimulus results in an increased net uptake of calcium (20, 21), which in turn brings about the discharge of stored hormone (21, 22). Interactions between the fluxes of Ca^{2+} and other ions, notably Na^{+}, may play an important regulatory role in this process (20). Our observations that calcium deficiency inhibited insulin release in response to sulphydryl reagents as well as to glucose are consistent with an obligatory requirement for calcium in the discharge mechanism. However, there was a striking difference between most of the sulphydryl reagents and glucose with regard to the magnitude of inhibition caused by calcium deficiency. Whereas the omission of calcium totally abolished even the initial response to glucose, the stimulatory effects of 6,6′-dithiodinicotinic acid, 5,5′-dithiobis(2-nitrobenzoic acid), and organic mercurials (1) were only partly inhibited. It therefore seems questionable whether the drastic effect of calcium deficiency on glucose-stimulated insulin release is due solely to inactivation of the discharge mechanism. It seems more likely that calcium is also required for the recognition of glucose or for a glucose-derived signal substance to induce the proper conformational change and ion permeability of the β-cell plasma membrane. Such a role for calcium in the early events of glucose stimulation might not be needed in the action of those sulphydryl reagents which have a direct, glucose-independent effect on the plasma membrane. From this point of view it is plausible that calcium deficiency has a drastic effect on insulin release in response to iodoacetamide (2), a reagent which acts as an insulin secretagogue only in the presence of glucose.

Although the idea that the sulphydryl reagents stimulate insulin release by modulating ionic fluxes may be the simplest explanation of the results obtained so far, alternative mechanisms cannot be ruled out. We have previously drawn attention to the possibility that sulphydryl reagents might displace insulin from the β-cell surface (1). It was recently reported that as large a molecule as peroxidase can attach to the β-cells and enter by endocytosis, the rate of entry being increased during glucose-stimulated insulin secretion (23). If there is a similar uptake of insulin during secretion, sulphydryl reagents could perhaps inhibit the uptake and thereby increase the net rate of insulin release

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