Iodoacetamide-Induced Sensitization of the Pancreatic β -Cells to Glucose Stimulation

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At a glucose concentration of 3 mM or less, iodoacetamide had no effect on the release of insulin from microdissected pancreatic islets of *ob/ob*-mice. At higher glucose concentrations, iodoacetamide exerted both an initial stimulatory and a subsequent inhibitory action. When islets were perfused with 1 mM-iodoacetamide and 17 mM-glucose the inhibitory action predominated after about 15 min of transient stimulation. With decreasing concentrations of iodoacetamide the stimulatory phase was gradually prolonged, and with 0.003–0.1 mM-iodoacetamide stimulation only was observed for 75 min. Prolonged stimulation was also noted after a short pulse of iodoacetamide. Similar responses to 0.1 mM-iodoacetamide were observed with islets from normal mice. With islets from *ob/ob*-mice the effect of 0.1 mM-iodoacetamide was reproduced with 0.1 mM-iodoacetate, whereas 0.1 mM-acetamide had no apparent effect. Iodoacetamide increased the $V_{\max}$ of glucose-stimulated insulin release without altering the apparent K_m for glucose. Leucine, glibenclamide or theophylline could not replace glucose in this synergistic action with iodoacetamide. Iodoacetamide rather inhibited the insulin-releasing action of theophylline. Iodoacetamide-induced potentiation of the glucose-stimulated insulin release was rapidly and reversibly inhibited by mannoheptulose, adrenaline, or calcium deficiency. The potentiating effect on insulin release was not paralleled by effects on glucose oxidation or on islet fructose 1,6-diphosphate. However, the inhibitory action of iodoacetamide might be explained by inhibition of glycolysis as evidenced by an inhibition of glucose oxidation and a rise of fructose 1,6-diphosphate. The results support our previous hypothesis that thiol reagents can stimulate insulin release by acting on relatively superficial thiol groups in the β -cell plasma membrane. Glycolysis seems to be necessary in order for iodoacetamide to stimulate in this way.

Iodoacetate and iodoacetamide, two thiol reagents, have been used to inhibit glucose metabolism (Hellerström *et al.*, 1970; Hellman *et al.*, 1971a; Ashcroft *et al.*, 1972c; Löffler *et al.*, 1972) and insulin release (Georg *et al.*, 1971; Kanazawa *et al.*, 1971; Löffler *et al.*, 1972) in the pancreatic islets. These effects have been tentatively attributed to inhibition of D-glyceraldehyde 3-phosphate-NAD oxidoreductase (phosphorylating) (EC 1.2.1.12) although there is general awareness of the possibility that iodoacetate and iodoacetamide at the concentrations used may not react specifically with this enzyme (see also Aleyassine, 1970). We have found that another class of thiol reagents, the organic mercurials, do not inhibit insulin release but act as strong insulin secretagogues (Bloom *et al.*, 1972). Small concentrations of chloromercuribenzenes-*p*-sulphonic acid, which penetrates cell membranes very slowly, caused a prompt release of insulin from microdissected islets exposed to this compound in a microperfusion system. For this and other reasons it was suggested that insulin release may be controlled by relatively superficial thiol groups in the β -cell plasma membrane (Bloom

et al., 1972). Our idea that reaction with these thiols causes insulin release is in apparent conflict with the inhibitory effects of iodoacetamide and iodoacetate reported by others. It was therefore decided to investigate in more detail the effects of iodoacetamide on the pancreatic β -cells. The work described here shows that iodoacetamide is not only an inhibitor of insulin release but can also act as an insulin secretagogue, depending on the concentration and the time of exposure to the thiol reagent. The stimulatory effect is most pronounced at low concentrations of iodoacetamide and requires that the β -cells be exposed to a certain concentration of glucose as well. Results are also given on the uptake of radioactive iodoacetamide by islets and on the effects of this compound on glucose transport, glucose metabolism and sucrose permeation in microdissected islets rich in β -cells.

Materials and Methods

Chemicals

A specially purified preparation of non-radioactive iodoacetamide (less than 0.005% free iodine) was

obtained from British Drug Houses Ltd., Poole, Dorset, U.K. Iodo[1- 14 C]acetamide, D-[6- 3 H]glucose, D-[U- 14 C]glucose, [6,6- 3 H]sucrose and [14 C]urea were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. 125 I-labelled insulin was from Novo A/S, Copenhagen, Denmark, and Farbwerke Hoechst A.G., Frankfurt/Main, Germany, and insulin antibodies were from Novo A/S and Wellcome Reagents Ltd., Beckenham, Kent, U.K. Non-radioactive sugars and ethanedioxybis-(ethylamine)tetra-acetic acid were from British Drug Houses Ltd. Non-radioactive urea as well as adrenaline, 5,5'-dithiobis-(2-nitrobenzoic acid), dithiothreitol, L-leucine and theophylline were from Sigma Chemical Co., St. Louis, Mo., U.S.A. Nicotinamide nucleotides, D-glyceraldehyde 3-phosphate ketol-isomerase, D-glyceraldehyde 3-phosphate-NAD oxidoreductase (phosphorylating) and ketose 1-phosphate aldehyde-lyase were from C.F. Boehringer und Soehne GmbH, Mannheim, Germany. N-4-[2-(5-chloro - 2 - methoxybenzamido)ethyl]benzenesulphonyl-N'-cyclohexylurea (glibenclamide) was from Farbwerke Hoechst A.G., and human serum albumin ('grade A') was from Kabi AB, Stockholm, Sweden. Crystalline mouse insulin was prepared by Novo A/S. Reagents of analytical grade and distilled de-ionized water were used throughout.

Methods

Animals and microdissection of islets. Adult obese-hyperglycemic mice (gene symbol *ob/ob*) of both sexes were obtained from the Umeå colony. Unless otherwise stated, *ob/ob* mice starved overnight were used. One series of experiment was performed with lean mice from the same colony. Fresh pancreatic islets were in all cases isolated by free-hand microdissection (Hellerström, 1964) in gassed ($O_2 + CO_2$, 95:5) Krebs-Ringer bicarbonate buffer (Umbreit *et al.*, 1964). The dissection medium contained glucose and albumin at the same concentrations as used during the subsequent incubation.

General aspects of incubations. All incubations were performed at 37°C. Unless otherwise stated, Krebs-Ringer bicarbonate buffer equilibrated with $O_2 + CO_2$ (95:5) and supplemented with 1 mg of albumin/ml was used as the basal medium. Most experiments began with a preliminary incubation period allowing the islets to equilibrate with the temperature and ingredients of the medium. The length of various incubation periods and the presence of additives to the basal medium are given in the legends to the Figures and Tables.

Insulin release. Two different techniques were used for studying insulin release. In some experiments batches of two islets were incubated in 300 μ l of medium for 60 min and insulin was determined in samples of medium taken at the end of incubation

(Lernmark, 1971). This technique will be referred to as 'batch-type' incubation. In other experiments, the dynamics of insulin release were studied with the aid of a microperfusion system that permits continuous sampling of insulin at various intervals during incubation. Two islets were placed in a small polythene-nylon chamber (4.5 μ l volume) and were perfused at a rate of 33–35 μ l/min. Two reservoirs were connected with the perfusion chamber so as to allow sudden alterations of the medium supplied to the perfused islets. In each perfusion experiment three different chambers were loaded with islets from a single animal and run in parallel. The results given represent mean values from these three chambers. The representative character of the published curves was confirmed in repeated experiments. Details of the microperfusion technique are given by Idahl (1972).

Insulin was assayed radioimmunologically. Free and bound insulin were separated by precipitation with ethanol (Heding, 1969). It was checked that iodoacetamide did not affect the determinations of immunoreactive insulin.

Glucose oxidation. Glucose oxidation over 1 h was measured by incubating single islets in liquid-scintillation vials equipped with a small glass centrewell (Keen *et al.*, 1963) containing 100 μ l of medium supplemented with 10 mM-D-[U- 14 C]glucose (1.7 mCi/mmol). Metabolism was stopped with HCl and $^{14}CO_2$ was collected in Hyamine and determined as previously described (Hellman *et al.*, 1971a). Essentially the same technique was used for measuring glucose oxidation over incubation periods as short as 15 min. In the latter case, however, batches of six islets were used in each vial to ensure the production of measurable amounts of $^{14}CO_2$. Before the islets were introduced into these vials, the medium was prewarmed to 37°C during continuous gassing with $O_2 + CO_2$ (95:5). In the 15-min experiments, each vial was equipped with a rubber stopper through which two needles attached to syringes were passed before incubation. The needles were kept well above the surface of the medium, and on one of them were threaded small bits of filter paper. To arrest metabolism, 0.1 M-HCl was introduced through the last-mentioned needle. The filter papers were then soaked with Hyamine injected through the other needle, and $^{14}CO_2$ was collected while the vials were shaken for 2 h at room temperature. In all glucose oxidation experiments blank values were obtained by incubating medium without islets.

Control experiments were carried out to test whether incubation with HCl affected the dry weight of islets. With the aid of an ocular micrometer 16 microdissected islets were matched with respect to size and divided into two groups. One group was incubated in albumin-free Krebs-Ringer bicarbonate medium, and one in the same kind of medium supplemented with 0.1 M-HCl. After 60 min of incubation,

the islets were freeze-dried and weighed. The dry weight of the eight control islets was $12.2 \pm 1.8 \mu\text{g}$, and that of the eight HCl-treated islets was $10.1 \pm 1.1 \mu\text{g}$ (mean values $\pm$ S.E.M.); these values are not statistically different.

Uptake of glucose, sucrose and iodoacetamide. All uptake experiments were based on the principle of double-labelling, by using one ^{14}C -labelled and one ^3H -labelled compound in each medium. Batches of three islets were incubated in each vial. The uptake of D-[6- ^3H]glucose or [6,6'- ^3H]sucrose was studied in relation to that of [^{14}C]urea, which equilibrates in the total islet water (Hellman *et al.*, 1971*b*). Since sucrose is normally restricted to the extracellular space of isolated islets (Hellman *et al.*, 1971*b,c*), the difference between the sucrose and urea spaces is negative in intact islets. The absolute value of this difference will be less under conditions of increased β -cell permeability to sucrose (Bloom *et al.*, 1972). Because of the rapid penetration of urea, the islet uptake of glucose in excess of the urea space is also negative at short incubation times. The absolute value of this deficit will increase under conditions of inhibited glucose transport (Bloom *et al.*, 1972). Throughout this paper the term 'space' is defined at any incubation time as (c.p.m. in islets)/(c.p.m. per unit volume of medium).

The uptake of iodo[1- ^{14}C]acetamide was studied in relation to that of [6,6'- ^3H]sucrose. By using sucrose as extracellular space marker, we have previously found that the intracellular water of incubated islets amounts to about 1.2 times their dry weight (Hellman *et al.*, 1971*b*). This makes it possible to predict the islet uptake of solutes that are uniformly distributed in the total tissue water: their uptake (expressed as mmol per kg dry weight of islets) should be 1.2 times the number of mmol per litre of incubation medium. On these grounds it will be shown that iodoacetamide is concentrated by isolated islets.

Details of the uptake experiments are given in the legends to the Figures and Tables. After freeze-drying and weighing, the islets were dissolved in Hyamine and analysed for ^3H and ^{14}C as previously described (Hellman *et al.*, 1971*b,c*).

Measurements of fructose 1,6-diphosphate. After incubation as described in Table 6, islets from fed mice were freeze-dried and weighed. Fructose 1,6-diphosphate was then measured by an enzymic fluorimetric technique employing oil wells and enzymic cycling, essentially as described by Matschinsky *et al.* (1968).

Weighing of islets and evaluation of results. In each experiment the islets were freeze-dried and weighed as previously described (Bloom *et al.*, 1972). This made it possible to express all results per unit dry weight of islet tissue. In batch-type experiments aimed at testing the effect of altered medium composition, notably the addition of iodoacetamide, parallel test and control incubations were performed

with islets isolated from the same animal. Statistical significances were then calculated from the mean differences between paired test and control incubations in a series of repeated experiments. The term 'experiment' refers to a set of incubations with islets from a single animal. Within each experiment all

Fig. 1. Dynamics of insulin release in response to iodoacetamide

(a) and (b) Islets were perfused with basal medium containing 17 mM-glucose. After 60 min the perfusion medium was suddenly switched to the same kind of medium supplemented with iodoacetamide. Exposure to iodoacetamide (bar) was continued for 75 min. Before perfusion with iodoacetamide, 1-min samples of effluent were taken for insulin assay. Continuous sampling was then used, over intervals of 1 or 2 min during the first 7 min and later over intervals of 5 or 10 min. The points represent the average rate of insulin release over each sampling period. The different curves are representative records of perfusion with different concentrations of iodoacetamide: (a) Δ , 0.1 mM-, $\blacksquare$, 0.3 mM-, $\bullet$, 1.0 mM-iodoacetamide; (b) Δ , 0.01 mM-, $\blacksquare$, 0.003 mM-, $\bullet$, 0.001 mM-iodoacetamide. For clarity, the results have been plotted on two diagrams of identical layout.

factors were usually tested in multiple incubations, the mean result of which was entered as one observation in the statistical treatment.

Results

Effects of iodoacetamide on insulin release

Dose-response relationships. Fig. 1 shows the dynamics of insulin release from islets perfused with 0.001–1.0 mM-iodoacetamide in the presence of 17 mM-glucose. Strikingly different results were obtained with the different concentrations of iodoacetamide. The effect of 1.0 mM-iodoacetamide was clearly biphasic. After an initial stimulation that lasted for about 15 min, insulin release steadily declined toward a rate that was lower than that caused by 17 mM-glucose alone. With decreasing concentrations of iodoacetamide the secretory response was gradually delayed and prolonged. An inhibitory effect of 0.3 mM-iodoacetamide was not observed until about 1 h of exposure of the islets to the thiol reagent. The secretory response to 0.1 mM-iodoacetamide was maintained at a high rate for as long as perfusion was continued. With concentrations of iodoacetamide as low as 0.01 mM or 0.003 mM, definite stimulation was still obtained but the release rate rose too slowly to reach a plateau within 1 h. When the concentration of iodoacetamide was lowered from 1.0 mM to 0.1 mM, both the length of the stimulatory phase and the maximum rate of insulin release increased.

These results show that iodoacetamide is both an inhibitor and a stimulator of insulin release. The net effect of iodoacetamide during a long period of conventional batch-type incubation will therefore depend on the balance between the stimulatory and inhibitory actions, and this will in turn depend on the incubation time and the concentration of iodoacetamide. This conclusion is statistically verified in Fig. 2, which summarizes the results of repeated batch-type incubations with different concentrations of iodoacetamide.

Reversibility of iodoacetamide action. The binding of iodoacetamide to thiol groups is not easily reversible (Webb, 1966). Therefore, if the insulin-releasing effect of iodoacetamide is due to the alkylation of thiol groups, it would be expected to persist for some time after withdrawal of the reagent. Fig. 3 shows that this is the case. When islets were continuously perfused with 17 mM-glucose, their exposure to 1.0 mM-iodoacetamide for 7 min resulted in a pronounced stimulation of insulin release, which reached its maximum about 30 min later. Similar results were obtained with 0.1 mM-iodoacetamide. A comparison of Figs. 1(a) and 3 reveals that the stimulatory effect of 1.0 mM-iodoacetamide was much more impressive after a brief pulse than during continuous perfusion with the thiol reagent. This indicates that the inhibitory effect of 1.0 mM-iodoacetamide differs from the

Fig. 2. Effects of different concentrations of iodoacetamide on the total insulin release over 60 min

After preliminary incubation of the islets for 40 min in basal medium containing 3 mM-glucose, batch-type incubations were performed for 60 min in media containing 17 mM-glucose as well as the indicated concentrations of iodoacetamide. Each point represents the mean value of eight different experiments. Bars denote S.E.M. Judging from the mean differences of paired observations, the stimulatory effect of 0.1 mM-iodoacetamide was significant with $P < 0.001$. The inhibitory effect of 1 mM-iodoacetamide was significant with $P < 0.01$.

stimulatory effect in requiring that the β -cells be exposed to the thiol reagent for a longer period of time.

Permissive action of glucose. In striking contrast to the pronounced stimulatory effect of 0.1 mM-iodoacetamide in the presence of 17 mM-glucose, 0.1 mM-iodoacetamide did not modify insulin release when the glucose concentration was only 3 mM (Fig. 4). Similarly, there was no significant effect of 0.1–10 mM-iodoacetamide in a series of batch-type incubations performed with 3 mM-glucose in the incubation media (not shown).

To test whether this permissive action is a specific attribute of glucose or is a general characteristic of insulin secretagogues, islets were incubated with 0.1 mM-iodoacetamide in the presence of 0.1 mM-glibenclamide, 20 mM-leucine or 5 mM-theophylline. Since the insulin-releasing actions of glibenclamide and theophylline are modified by glucose, these tests

Fig. 3. Dynamics of insulin release in response to a short pulse of iodoacetamide

Islets were perfused with basal medium containing 17 mM-glucose. After 60 min the perfusion medium was suddenly switched to the same kind of medium containing 1.0 mM-iodoacetamide. Perfusion with iodoacetamide (bar) lasted for only 7 min, after which the experiment was continued with the initial, iodoacetamide-free medium. Insulin was sampled and results were expressed as in Fig. 1.

Fig. 4. Synergism between iodoacetamide and glucose. Islets were perfused with 0.1 mM-iodoacetamide (indicated by the bar) as described in Fig. 1, except that the perfusion medium contained either 3 mM-glucose (○) or 17 mM-glucose (●). These concentrations of glucose were also present during the preliminary perfusion for 60 min with iodoacetamide-free medium. In addition, the results of perfusion with 0.1 mM-iodoacetamide in the presence of 17 mM-glucose is shown (△). This control experiment was designed in the same way as the corresponding experiment with iodoacetamide. Insulin was sampled and results were expressed as in Fig. 1.

were performed both at 3 mM-glucose and at 8 mM-glucose. Table 1 shows that at 3 mM-glucose iodoacetamide significantly stimulated insulin release

when glibenclamide or leucine were present. Iodoacetamide had no effect in the presence of 5 mM-theophylline. Although iodoacetamide did not significantly stimulate insulin release in the presence of 3 mM-glucose alone, the mean value was somewhat increased in the iodoacetamide group. When this is taken into account, the synergism between iodoacetamide and glibenclamide or leucine is doubtful: the effects of glibenclamide and leucine were not significantly larger with 0.1 mM-iodoacetamide than in the absence of iodoacetamide.

The conclusion that glibenclamide or leucine cannot replace glucose in enabling iodoacetamide to stimulate insulin release is encouraged by the results obtained at 8 mM-glucose. Whereas glibenclamide ($P < 0.05$), leucine ($P < 0.025$) and theophylline ($P < 0.02$) stimulated insulin release in the absence of iodoacetamide, none of them did so when 0.1 mM-iodoacetamide was present. In fact there was a negative interaction between iodoacetamide and theophylline, since iodoacetamide significantly inhibited the secretory response to theophylline in the presence of 8 mM-glucose.

Since the insulin-releasing action of glucose is inhibited by mannoheptulose (Coore & Randle, 1964; Hellman *et al.*, 1972a), we decided to investigate whether mannoheptulose can also inhibit the permissive action of glucose on iodoacetamide-stimulated insulin release. Fig. 5 shows that this is the case. This result suggests that the synergism between glucose and iodoacetamide is intimately related to the mechanisms by which glucose normally stimulates insulin release.

Another important aspect of the glucose-dependent nature of iodoacetamide-stimulated insulin release is shown in Fig. 6. During continuous perfusion of islets with 0.1 mM-iodoacetamide, marked stimulation of insulin release was obtained only as long as 17 mM-glucose was also present in the medium. Immediately after the withdrawal of glucose, the release rate quickly declined in spite of continued perfusion with iodoacetamide. When 17 mM-glucose was again included in the medium, the rate of insulin release rose almost instantaneously to the high value recorded before the supply of glucose was interrupted. Thus the permissive effect of glucose is as easily reversible as is the insulin-releasing action of glucose alone (Grotsky *et al.*, 1970).

The effect of different glucose concentrations on the stimulatory action of 0.1 mM-iodoacetamide is shown in Fig. 7. The response to iodoacetamide increased progressively with rising glucose concentration. The general appearance of the curves suggests that iodoacetamide increases the V_{max} of glucose-stimulated insulin release with little or no effect on the apparent K_m .

The permissive action of glucose could mean that glucose in one way or another alters the ability of

Table 1. *Effects of glibenclamide, leucine and theophylline on the insulin-releasing capacity of iodoacetamide*

After preliminary incubation of the islets for 40 min in basal medium containing 3 mM-glucose, batch-type incubations were performed for 60 min in media containing glucose, iodoacetamide and other test substances as indicated. Results are given as mean values $\pm$ S.E.M. for the numbers of experiments stated in parentheses. Effects of iodoacetamide were judged from the mean differences between paired incubations in the presence and absence of the thiol reagent: * $P < 0.02$; ** $P < 0.005$.

Test substance	Insulin released (ng/h per μ g dry wt. of islets)	
	0 mM-Iodoacetamide	0.1 mM-Iodoacetamide
Incubations in 3 mM-glucose		
None (control)	0.48 ± 0.15 (7)	0.84 ± 0.34 (7)
L-Leucine (20 mM)	1.69 ± 0.42 (7)	$2.54 \pm 0.43^{**}$ (7)
Theophylline (5 mM)	1.87 ± 0.89 (7)	1.12 ± 0.37 (7)
None (control)	0.49 ± 0.11 (7)	1.08 ± 0.28 (7)
Glibenclamide (0.1 mM)	1.05 ± 0.15 (7)	$2.32 \pm 0.37^*$ (7)
Incubations in 8 mM-glucose		
None (control)	1.72 ± 0.25 (6)	$4.64 \pm 0.61^{**}$ (6)
L-Leucine (20 mM)	3.79 ± 0.53 (6)	5.15 ± 0.54 (6)
Theophylline (5 mM)	8.16 ± 1.77 (6)	$4.70 \pm 1.32^*$ (6)
Glibenclamide (0.1 mM)	3.04 ± 0.83 (6)	4.18 ± 0.73 (6)

Fig. 5. *Inhibitory action of mannoheptulose on iodoacetamide-induced insulin release*

After preliminary perfusion for 45 min with basal medium containing 17 mM-glucose, the islets were suddenly exposed to the same kind of medium supplemented with 0.1 mM-iodoacetamide. Perfusion with 0.1 mM-iodoacetamide and 17 mM-glucose (hatched bar) was continued for 90 min. However, 45 min after the islets were first exposed to iodoacetamide, 20 mM-mannoheptulose was also included in the medium. The perfusion with mannoheptulose (cross-hatched bar) lasted for 25 min. Insulin was sampled continuously during perfusion with iodoacetamide, and each point represents the average rate of insulin release over the respective sampling period.

Fig. 6. *Reversibility of the permissive action of glucose on iodoacetamide-induced insulin release*

After preliminary perfusion for 45 min with basal medium containing 17 mM-glucose, the islets were suddenly exposed to the same kind of medium supplemented with 0.1 mM-iodoacetamide. Perfusion with iodoacetamide (hatched bar) was continued for 90 min. However, 45 min after the islets were first exposed to iodoacetamide, glucose was omitted from the medium. The perfusion with iodoacetamide in glucose-free medium lasted for 25 min, after which the medium was again suddenly switched to one containing both iodoacetamide and 17 mM-glucose. The presence of 17 mM-glucose is indicated by open bars. Insulin was sampled and results were expressed as described in Fig. 5.

Fig. 7. Effects of different concentrations of glucose on iodoacetamide-induced insulin release

After preliminary incubation of the islets for 40 min in basal medium containing 3 mM-glucose, batch-type incubations were performed for 60 min in media containing glucose as indicated. Parallel incubations were performed in media containing no further additives (○) and in media also containing 0.1 mM-iodoacetamide (●). Each point represents the mean value of 8–15 different experiments. Bars denote S.E.M.

β -cells to react with iodoacetamide. Alternatively, iodoacetamide might react with the β -cells independently of glucose and through this reaction make the β -cells more responsive to a subsequent glucose stimulus. To investigate this problem we have exploited the observation that the effect of iodoacetamide persists for a long time after withdrawal of the thiol reagent (cf. Fig. 3). Table 2 summarizes the outcome of experiments in which the islets were pre-treated with different combinations of glucose and iodoacetamide before the insulin-releasing action of glucose was measured in the absence of thiol reagent. The results show that glucose need not be present for iodoacetamide to make the β -cells hyper-responsive to subsequent stimulation with glucose.

Effects of calcium deficiency and adrenaline. The insulin-releasing effect of glucose alone is strongly inhibited by calcium deficiency and by adrenaline (Coore & Randle, 1964; Grodsky & Bennett, 1966; Lernmark & Hellman, 1970). Similarly, the stimulatory effect of 0.1 mM-iodoacetamide was abolished when calcium was removed from (Fig. 8) or adrenaline was added to (Fig. 9) the perfusion medium.

Fig. 8. Effect of calcium deficiency on iodoacetamide-induced insulin release

After preliminary perfusion for 45 min with basal medium containing 17 mM-glucose, the islets were suddenly exposed to the same kind of medium supplemented with 0.1 mM-iodoacetamide. Perfusion with 0.1 mM-iodoacetamide and 17 mM-glucose (hatched bar) was continued for 90 min. However, 45 min after the islets were first exposed to iodoacetamide, the basal composition of the medium was altered by substituting NaCl for CaCl_2 and by adding 0.5 mM-ethanedioxybis(ethylamine)tetra-acetic acid. Perfusion with the calcium-deficient medium (cross-hatched bar) lasted for 25 min, after which the perfusion was continued with medium of normal basal composition. Insulin was sampled and results were expressed as described in Fig. 5.

Fig. 9. Effect of adrenaline on iodoacetamide-induced insulin release

After preliminary perfusion for 45 min with basal medium containing 17 mM-glucose, the islets were suddenly exposed to the same kind of medium supplemented with 0.1 mM-iodoacetamide. Perfusion with 0.1 mM-iodoacetamide and 17 mM-glucose (hatched bar) was continued for 90 min. However, 45 min after the islets were first exposed to iodoacetamide, 2 μg of adrenaline/ml was also included in the medium. Perfusion with adrenaline (cross-hatched bar) lasted for 25 min. Insulin was sampled and results were expressed as described in Fig. 5.

Table 2. *Effects of pretreatment with glucose and iodoacetamide on the subsequent secretory response to glucose*

Each individual batch of islets was subjected to four consecutive incubations. During three periods of 15 min the islets were pretreated with different combinations of glucose and iodoacetamide as indicated in the top section of the Table. Four combinations were used, denoted groups 1-4. After pretreatment, the islets were incubated for 60 min in iodoacetamide-free medium containing either 0 mM- or 20 mM-glucose. The amounts of insulin released during this 60-min incubation are given in the middle section of the Table. The bottom section of the Table shows a statistical analysis of whether the enhancing action of iodoacetamide requires that glucose be present when the islets are exposed to the thiol reagent. Results are given as mean values $\pm$ S.E.M. for seven different experiments.

Test substances present during pretreatment			
Group	First 15-min period	Second 15-min period	Third 15-min period
1	None	None	None
2	None	0.1 mM-Iodoacetamide	None
3	20 mM-Glucose	20 mM-Glucose	None
4	20 mM-Glucose	20 mM-Glucose plus 0.1 mM-iodoacetamide	None

Insulin released during incubation for 60 min (ng/h per μ g dry wt. of islets)			
	0 mM-glucose	20 mM-glucose	<i>P</i> value for effect of glucose
1	0.55 $\pm$ 0.11	2.96 $\pm$ 0.70	<0.01
2	0.79 $\pm$ 0.13	6.72 $\pm$ 0.74	<0.001
3	0.52 $\pm$ 0.21	3.56 $\pm$ 0.49	<0.001
4	1.12 $\pm$ 0.39	9.65 $\pm$ 1.38	<0.001

Increment of insulin release in response to 20 mM-glucose (ng/h per μ g dry wt. of islets)			
	Pretreatment with 0 mM-iodoacetamide	Pretreatment with 0.1 mM-iodoacetamide	<i>P</i> value for effect of iodoacetamide
1 and 2	2.41 $\pm$ 0.65	5.93 $\pm$ 0.65	<0.005
3 and 4	3.04 $\pm$ 0.45	8.53 $\pm$ 1.33	<0.01

Fig. 10. *Synergism between iodoacetate and glucose*

Islets were perfused with 0.1 mM-iodoacetate (indicated by the bar) in the presence of 3 mM-glucose (○) or 17 mM-glucose (●). The experimental design was the same as that described for perfusion with 0.1 mM-iodoacetamide in Fig. 4.

Comparisons of iodoacetamide with iodoacetate and acetamide. If iodoacetamide stimulates insulin release by reacting with thiol groups, a similar stimulation would be expected with iodoacetate but not with acetamide. Fig. 10 shows that 0.1 mM-iodoacetate potentiated glucose-stimulated insulin release but had no effect in the presence of 3 mM-glucose. Fig. 4 shows that 0.1 mM-acetamide did not appreciably potentiate glucose-stimulated insulin release.

Effect of iodoacetamide on islets from lean mice. The β -cells of *ob/ob* mice respond in the same way as those of normal mice to all investigated modifiers of insulin release. Therefore there would be little reason for assuming that the effects of iodoacetamide are restricted to the *ob/ob* variety. Nevertheless, since a substantial amount of work is being performed with islets isolated from lean mice, it was checked that they are also stimulated by 0.1 mM-iodoacetamide (Fig. 11).

Uptake of iodoacetamide by islets

As shown in Fig. 12, the islets accumulated iodo-[1-¹⁴C]acetamide in amounts several times larger

Fig. 11. Iodoacetamide-induced potentiation of insulin release from islets of lean mice

Except that a different type of islets was used, the experiment was designed as described in Fig. 1. Perfusion with 0.1 mM-iodoacetamide is indicated by the bar.

than those that can be accounted for by passive distribution in the tissue water. When islets were preloaded with iodo[1-¹⁴C]acetamide and then transferred to an iodoacetamide-free medium, there was a very slow loss of ¹⁴C from the islets (Fig. 12). Dithiothreitol, a thiol protector, and 5,5'-dithiobis-(2-nitrobenzoic acid), an oxidant of thiol groups, significantly inhibited the 45 min uptake of iodoacetamide (Table 3). These results strongly suggest that iodoacetamide was bound to islet thiol groups in a relatively irreversible manner. Glucose had no measurable effect on the uptake of iodoacetamide by islets.

Effects of iodoacetamide on glucose metabolism

Glucose oxidation. At a concentration of 1.0 mM, iodoacetamide drastically inhibited the production of ¹⁴CO₂ from islets incubated for 60 min with 10 mM-D-[U-¹⁴C]glucose (Table 4). No significant effects were obtained with 0.1 mM- or 0.01 mM-iodoacetamide. However, with 0.01 mM-iodoacetamide there was a tendency to stimulated glucose oxidation, and with 0.001 mM-iodoacetamide the stimulatory effect was significant at the 5 % level. Since it seems possible that the effect of iodoacetamide on glucose oxidation might be multiphasic with time, a stimulatory action of iodoacetamide might perhaps be obscured in experiments based on incubation for as long as 60 min.

Table 3. Effects of glucose, dithiothreitol and 5,5'-dithiobis-(2-nitrobenzoic acid) on the islet uptake of iodoacetamide

Islets were subjected to a preliminary incubation for 45 min in albumin-free basal medium supplemented with 3 mM-glucose and one of the listed test substances. They were then incubated for either 5 or 45 min in albumin-free basal medium supplemented with the same test substance as used during the preliminary incubation as well as with 0.2 mM-iodo[1-¹⁴C]acetamide (58 mCi/mmol) and 0.1 mM-[6,6'-³H]sucrose (150 mCi/mmol). The values denote islet uptake of iodoacetamide corrected for ¹⁴C in the sucrose space. Results are given as mean values ± S.E.M. for each test substance as well as for the differences between paired test and control incubations. Statistical differences were judged from these differences: **P* < 0.005. The numbers of experiments are stated within parentheses.

Test substance	Uptake of iodoacetamide (mmol per kg dry wt. of islets)	
	Test minus control	
Incubation for 5 min		
None (control)	2.21 ± 0.12 (4)	—
Glucose (20mM)	2.41 ± 0.19 (4)	0.20 ± 0.14 (4)
Dithiothreitol (1 mM)	2.51 ± 0.58 (4)	0.30 ± 0.70 (4)
5,5'-Dithiobis- (2-nitrobenzoic acid) (1 mM)	2.06 ± 0.18 (4)	−0.15 ± 0.18 (4)
Incubation for 45 min		
None (control)	9.65 ± 0.90 (6)	—
Glucose (20mM)	8.87 ± 0.70 (6)	−0.78 ± 0.62 (6)
Dithiothreitol (1 mM)	3.10 ± 0.60 (6)	−6.55 ± 1.22* (6)
5,5'-Dithiobis- (2-nitrobenzoic acid) (1 mM)	7.11 ± 0.63 (6)	−2.54 ± 0.40* (6)

Fig. 12. Islet uptake and release of iodoacetamide with time

In studies of iodoacetamide uptake (a), the islets were first incubated for 45 min in albumin-free basal medium containing 3 mM-glucose. They were then incubated for different periods of time in albumin- and glucose-free basal medium supplemented with 0.2 mM-iodo[1-¹⁴C]acetamide (58 mCi/mmol) and 0.1 mM-[6,6-³H] sucrose (150 mCi/mmol). Each point represents the islet uptake of iodoacetamide after correction for label in the sucrose (extracellular) space. Results are given as mean values $\pm$ S.E.M. for four to six different experiments. Note that under the conditions used, passive distribution of iodoacetamide in the β -cell water would account for only 0.24 mmol/kg dry wt. of islets (cf. Hellman *et al.*, 1971b). In studies of iodoacetamide release (b), the islets were first incubated for 30 min in albumin-free basal medium supplemented with 0.2 mM-iodo[1-¹⁴C]acetamide (58 mCi/mmol). They were then incubated for different periods of time in the same kind of medium without iodoacetamide. The amounts of iodoacetamide retained by the islets are given as mean values $\pm$ S.E.M. for five to nine different experiments.

Table 4. Effects of iodoacetamide on glucose oxidation

After preliminary incubation for 45 min in basal medium containing 3 mM-glucose, the islets were incubated for 60 min in media containing 10 mM-D-[U-¹⁴C]glucose (1.7 mCi/mmol) and iodoacetamide as indicated. No albumin was present in the media except in the test of 0.001 mM-iodoacetamide, when both test and control media contained 1 mg of albumin/ml. Amounts of ¹⁴CO₂ produced are expressed as mmol of glucose equivalents oxidized/h per kg dry wt. of islets. Results are given as mean values $\pm$ S.E.M. for the numbers of experiments stated in parentheses. In addition to the values obtained for each medium, the differences between paired test and control incubations are presented. Statistical significances were judged from these differences: * P < 0.05; ** P < 0.005.

Concn. of iodoacetamide (mM)	Rate of oxidation (mmol/h per kg dry wt. of islets)	
		Test minus control
Nil (control)	31.7 $\pm$ 4.7 (5)	—
0.001	36.8 $\pm$ 6.2 (5)	5.1 $\pm$ 1.8* (5)
Nil (control)	25.3 $\pm$ 3.6 (6)	—
0.01	31.4 $\pm$ 4.3 (6)	6.1 $\pm$ 4.0 (6)
0.1	25.9 $\pm$ 2.9 (6)	0.6 $\pm$ 4.9 (6)
Nil (control)	28.3 $\pm$ 4.8 (6)	—
1.0	4.7 $\pm$ 1.2 (6)	-23.6 $\pm$ 4.3** (6)

Table 5. *Effect of 0.01 mM-iodoacetamide on glucose oxidation with time*

Islets were subjected to a preliminary incubation for 30min in basal medium containing 3 mM-glucose. They were then incubated for either 0, 15, 30, or 45 min in basal medium containing 10 mM-non-radioactive glucose and 0.01 mM-iodoacetamide. Finally, the islets were incubated for 15 min in a medium that had the same chemical composition but contained 10 mM-D-[U- 14 C]glucose (1.7 mCi/mmol) instead of non-radioactive glucose. The rates of glucose oxidation during this last 15 min period are expressed as in Table 4. Results are given as mean values $\pm$ S.E.M. for each group as well as for the differences between paired test and control incubations. Statistical significances were judged from these differences: * $P < 0.005$. The numbers of experiments are given in parentheses. The given rates (mmol/15 min) are not directly comparable with those in Table 4 (mmol/h). Each period of $^{14}\text{CO}_2$ production includes an initial lag phase, which influences the results proportionally more in 15 min incubations.

Time of exposure to iodoacetamide (min)	Rate of oxidation (mmol/15 min per kg dry wt. of islets)		
	Control (a)	Test (b)	(b) - (a)
15	4.36 $\pm$ 0.32 (3)	3.92 $\pm$ 0.59 (3)	-0.44 $\pm$ 0.59 (3)
30	4.41 $\pm$ 0.48 (3)	4.60 $\pm$ 0.59 (3)	0.19 $\pm$ 0.55 (3)
45	3.96 $\pm$ 0.26 (3)	4.74 $\pm$ 0.17 (3)	0.78 $\pm$ 0.22 (3)
60	4.55 $\pm$ 0.55 (4)	3.33 $\pm$ 0.68 (4)	-1.22 $\pm$ 0.14* (4)

Table 6. *Effect of iodoacetamide on the islet content of fructose 1,6-diphosphate*

After preliminary incubation for 10 min in basal medium supplemented with 3 mM-glucose, islets from fed mice were incubated for 40 min in basal medium supplemented with 17 mM-glucose as well as the listed concentrations of iodoacetamide. All media also contained 5 mg of albumin/ml. Results are given as mean values $\pm$ S.E.M. for the numbers of experiments stated in parentheses. Statistical significances were judged from the differences between paired test and control incubations: * $P < 0.02$; ** $P < 0.001$.

Concn. of iodoacetamide (mM)	Islet content of fructose 1,6-diphosphate (μ mol/kg dry wt. of islets)	
	Control	Iodoacetamide
0.10	450 $\pm$ 83 (6)	410 $\pm$ 79 (6)
0.25	413 $\pm$ 65 (10)	714 $\pm$ 64* (10)
0.50	388 $\pm$ 50 (7)	7933 $\pm$ 748** (7)

To examine this possibility, one series of experiments was performed in which the incubations were divided into 15 min periods. Iodoacetamide was tested at a concentration of 0.01 mM, since this concentration both caused a marked stimulation of insulin release (Fig. 1) and tended to stimulate glucose oxidation over 60 min (Table 4). As shown in Table 5, 0.01 mM-iodoacetamide did not stimulate glucose oxidation to any great extent during the four consecutive 15 min incubations. On the contrary, glucose oxidation was significantly inhibited during the last 15 min of a 1 h period of exposure to 0.01 mM-iodoacetamide.

Islet content of fructose 1,6-diphosphate. The previously reported (Hellerström *et al.*, 1970; Georg *et al.*, 1971; Hellman *et al.*, 1971a; Kanazawa *et al.*, 1971; Ashcroft *et al.*, 1972c; Löffler *et al.*, 1972) inhibitory effects of iodoacetate and iodoacetamide

on islet metabolism and insulin release have tentatively been attributed to inhibition of D-glyceraldehyde 3-phosphate-NAD oxidoreductase (phosphorylating). This is a well-known locus for the inhibition of glycolysis by thiol reagents in several tissues (Webb, 1966), but little is known about its sensitivity to iodoacetamide in the pancreatic β -cells. It was therefore decided to investigate the effects of different concentrations of iodoacetamide on the islet content of a glycolytic intermediate located above this metabolic step. Table 6 shows that incubation for 40 min with 0.1 mM-iodoacetamide had no significant effect on the islet content of fructose 1,6-diphosphate. However, 0.25 mM-iodoacetamide brought about a doubling of the fructose 1,6-diphosphate, and with 0.5 mM-iodoacetamide the content of this metabolite was increased about 20-fold.

Table 7. *Effect of iodoacetamide on glucose transport and sucrose space*

Islets were subjected to a preliminary incubation for 45 min in albumin-free basal medium containing 3 mM-glucose and iodoacetamide as indicated. In studies of glucose transport, they were then incubated for 45 s in albumin-free basal medium supplemented with 2.0 mM-D-[6-³H]glucose (10 mCi/mmol) and 0.5 mM-[¹⁴C]urea (20 mCi/mmol). In studies of sucrose space, incubation was performed for 15 min in basal medium supplemented with 0.5 mM-[6,6'-³H]sucrose (30 mCi/mmol) as well as 0.5 mM-[¹⁴C]urea (20 mCi/mmol). During incubations with radioactive sugars the media also contained the same concentrations of iodoacetamide as during the preliminary incubation. The values denote islet uptake of glucose or sucrose in excess of the urea space. Results are given as mean values $\pm$ S.E.M. for each group as well as for the differences between paired test and control incubations. The numbers of experiments are stated within parentheses.

Concn. of iodoacetamide (mM)	Sugar uptake in excess of urea space (mmol per kg dry wt. of islets)	
	Test minus control	
Incubations with D-[6- ³ H]glucose		
Nil (control)	-1.23 ± 0.16 (6)	—
0.01	-1.16 ± 0.21 (6)	0.07 ± 0.16 (6)
0.1	-0.82 ± 0.24 (6)	0.41 ± 0.33 (6)
1.0	-1.10 ± 0.22 (6)	0.13 ± 0.15 (6)
Incubations with [6,6'- ³ H]sucrose		
Nil (control)	-1.34 ± 0.04 (5)	—
0.01	-1.34 ± 0.02 (5)	0.00 ± 0.04 (5)
0.1	-1.30 ± 0.03 (5)	0.04 ± 0.03 (5)
1.0	-1.19 ± 0.08 (5)	0.15 ± 0.07 (5)

Effect of iodoacetamide on glucose transport and sucrose space

Table 7 shows that 0.01–1.0 mM-iodoacetamide had no significant effect on the 45 s uptake of glucose or the 15 min uptake of sucrose by isolated islets.

Discussion

The major conclusion to be drawn from the present results is that iodoacetamide is both an inhibitor and a potentiator of glucose-stimulated insulin release. The net effect depends on several factors such as the incubation time, the concentration of iodoacetamide, and the accompanying concentration of glucose. Great caution must therefore be exercised when interpreting the overall effects of iodoacetamide in conventional batch-type incubations. For example, were it not for the dynamic records in Fig. 1, the results in Fig. 2 might erroneously have been interpreted as showing that 0.3 mM-iodoacetamide has no effect on insulin release. In a batch-type experiment with isolated rat islets, Löffler *et al.* (1972) noted that 0.5 mM-iodoacetate had a stronger inhibitory effect on glucose oxidation than on insulin release. Matschinsky *et al.* (1972) used this finding as one of several arguments in favour of the idea that glucose does not stimulate insulin release only through its

metabolism. Although this idea may in itself be correct, the present results make it seem hazardous to draw conclusions from the overall effects of iodoacetate in batch-type incubations.

The discovery that iodoacetamide is both a potentiator and an inhibitor of insulin release resolves the apparent conflict which initiated the present study, i.e. that there are previous reports of iodoacetate- and iodoacetamide-inhibited β -cell function (Hellerström *et al.*, 1970; Georg *et al.*, 1971; Hellman *et al.*, 1971a; Kanazawa *et al.*, 1971; Ashcroft *et al.*, 1972c; Löffler *et al.*, 1972), whereas insulin release is markedly stimulated by the thiol reagents *p*-chloromercuribenzoic acid and chloromercuribenzenep-sulphonic acid (Bloom *et al.*, 1972). It is now clear that the insulin-releasing capacity of thiol reagents is not restricted to the reversibly acting organic mercurials but is shared by an alkylating compound with a virtually irreversible action. Although iodoacetamide may not react specifically with thiol groups in the β -cells, several observations indicate that the insulin-releasing effect is due to the alkylation of thiol groups: (1) The effect of iodoacetamide was reproduced with iodoacetate but not with acetamide. (2) Potentiation of insulin release was not quickly reversed on withdrawal of iodoacetamide. (3) Islet uptake of iodoacetamide also was slowly reversible and was inhibited by dithiothreitol and 5,5'-dithiobis-(2-nitrobenzoic acid).

If, as we have suggested, the regulatory thiol groups are located rather superficially in the β -cell plasma membrane (Bloom *et al.*, 1972), this may explain the dual and biphasic effect of iodoacetamide on insulin release. The inhibitory action of iodoacetamide was preceded by the stimulatory phase and was gradually delayed at decreasing iodoacetamide concentrations, which enhanced the stimulatory response. This could mean that iodoacetamide exerts its stimulatory and inhibitory actions at different loci in the β -cells. It has been suggested (Georg *et al.*, 1971; Kanazawa *et al.*, 1971) that iodoacetate inhibits insulin release by inhibiting D-glyceraldehyde 3-phosphate-NAD oxidoreductase (phosphorylating). Our results with iodoacetamide are consistent with this view. The inhibitory effect on insulin release was concentration-dependent in a way that agreed well with the effects of different concentrations of iodoacetamide on the islet content of fructose 1,6-diphosphate as well as on glucose oxidation. It therefore seems reasonable to assume that the initial stimulation of insulin release, presumably caused by the alkylation of superficial thiol groups, was overcome by an inhibition of glycolysis when iodoacetamide had penetrated more deeply into the β -cells. In this way we can explain not only the dual effect of continuous perfusion with 1.0mM-iodoacetamide but also why the stimulatory phase predominated at low iodoacetamide concentrations and after a brief pulse of 1.0mM-iodoacetamide.

Since thiol reagents can increase the permeability of plasma membranes (Watkins *et al.*, 1970, 1971; Rothstein, 1970), it is theoretically possible that some such agent could cause passive diffusion of insulin out of leaky β -cells. However, there is no reason to believe that the iodoacetamide-induced insulin release was of this artificial nature. Insulin release was stimulated at concentrations of iodoacetamide that had no significant effects on glucose transport or on the sucrose space, two indicators of membrane function. Like insulin release in response to glucose (Coore & Randle, 1964; Grodsky & Bennett, 1966; Lernmark & Hellman, 1970) and organic mercurials (Bloom *et al.*, 1972), that elicited by iodoacetamide was strongly inhibited by calcium deficiency and by adrenaline. Most important, the insulin-releasing action of iodoacetamide was only seen at glucose concentrations higher than 3mM, although glucose had no measurable effect on the islet uptake of iodoacetamide.

The permissive action of glucose on iodoacetamide-induced insulin release resembles the insulin-releasing action of glucose alone in being inhibited by mannoheptulose and in being rapidly reversible on withdrawal of glucose. Perhaps the effect of iodoacetamide is therefore best described as a sensitization of the β -cells to glucose. This sensitization was reflected in an increased $V_{\max}$ of glucose-stimulated

insulin release, whereas iodoacetamide seemed to have no effect on the apparent K_m . These observations suggest that iodoacetamide somehow amplified the secretagogic signal aroused by glucose. Since the nature of the glucose-aroused signal is unknown, it is not possible to define in precise molecular terms the mechanisms that may be responsible for this amplification. Note, however, that the present results give no indication of whether glucose stimulates insulin release through its metabolism or by directly acting on a membrane-located glucose receptor or both (Grodsky *et al.*, 1963; Coore & Randle, 1964; Hellman, 1970; Ashcroft *et al.*, 1972a; Hellman *et al.*, 1972b,c; Matschinsky *et al.*, 1972). Although the marked insulin-releasing action of iodoacetamide was not paralleled by any corresponding enhancement of glucose metabolism, it does not follow that glucose metabolism was not rate-limiting for the permissive effect of glucose. It is as conceivable that iodoacetamide amplified the secretagogic effect of a signal substance arising from glucose metabolism as that it amplified some other kind of signal arising from the binding of glucose to a direct receptor.

It has been suggested that the insulin-releasing action of glucose can be potentiated by stimulation of adenylate cyclase and inhibition of cyclic nucleotide phosphodiesterase (Malaisse *et al.*, 1967; Montague & Cook, 1971; Ashcroft *et al.*, 1972b). Such potentiation has been attributed to a capacity of 3':5'-cyclic AMP to amplify the effect of glucose on the β -cell content of Ca^{2+} (Brisson *et al.*, 1972). Although little is known about the regulation of 3':5'-cyclic AMP in β -cells, it seems well established that α -adrenergic blocking agents as well as β -adrenergic stimuli enhance the release of insulin (Malaisse *et al.*, 1967; Loubatières *et al.*, 1971). It is therefore noteworthy that thiol reagents have been reported to inhibit the function of α -adrenergic receptors in lizard melanophores (Goldman & Hadley, 1972). However, it seems fairly unlikely that iodoacetamide stimulates insulin release by increasing the islet content of 3':5'-cyclic AMP. The stimulatory effect of theophylline, which is usually attributed to inhibition of cyclic nucleotide phosphodiesterase (Malaisse *et al.*, 1967; Montague & Cook, 1971; Ashcroft *et al.*, 1972b), was not enhanced, but significantly inhibited, by iodoacetamide. Neither was there any demonstrable synergism between iodoacetamide, on the one hand, and leucine or glibenclamide on the other. This would suggest that iodoacetamide does not affect any of those components of the secretory machinery that are involved in the action of most insulin secretagogues.

The marked glucose dependence of iodoacetamide as an insulin secretagogue contrasts with our previous observations on the insulin-releasing action of organic mercurials. *p*-Chloromercuribenzoic acid as well as chloromercuribenzene-*p*-sulphonic acid were strongly stimulatory at both 3mM- and 17mM-glucose, the

effects of organic mercurials and glucose apparently being additive (Bloom *et al.*, 1972). The reasons for this difference between iodoacetamide and organic mercurials are obscure. Further experiments are necessary to establish whether it reflects different modes of reacting with thiol groups or whether in fact different thiol groups are involved in the stimulatory actions of iodoacetamide and organic mercurials. As emphasized by Rothstein (1970), the effects of thiol reagents on cell membranes are determined not only by their chemical reactivity but also by their ability to reach various targets in the membrane.

Iodoacetamide is not the only thiol reagent whose effect on the β -cell is markedly influenced by glucose. The β -cells differ from most other cells in extensively concentrating alloxan (Hammarström *et al.*, 1966) and in being particularly vulnerable to the cytotoxic action of this compound. Lazarow (1949) suggested that alloxan kills the β -cells by reacting with thiol groups which may be located in the β -cell plasma membrane (Cooperstein *et al.*, 1964). Interestingly, the diabetogenic effect of alloxan can be blocked by glucose (Bhattacharya, 1954), and this blocking can in turn be prevented with mannoheptulose (Scheynius & Täljedal, 1971). Glucose is known to alter drastically the membrane potential of pancreatic β -cells, almost certainly by altering the conformation of the plasma membrane in such a way as to modulate ionic fluxes selectively (Matthews & Dean, 1970). It has been suggested that the glucose-induced conformation is unfavourable for a reaction between alloxan and the membrane (Scheynius & Täljedal, 1971). The antagonistic relationship between alloxan and glucose thus contrasts with the striking synergism between iodoacetamide and glucose reported here. This may be a further reason for assuming that the function of β -cells depends on more than one class of membrane thiol groups with different reactivity towards exogenous reagents. Havu (1969) suggested that alloxan is β -cytotoxic because it is capable of reacting with dithiols; iodoacetamide is a monothiol reagent.

Treatment with iodoacetamide or iodoacetate *in vivo* is known to influence the blood glucose concentration. Both hyperglycaemic and hypoglycaemic effects have been reported, which is not surprising in view of the manifold effects of thiol reagents in the intact animal (Webb, 1966). It is particularly noteworthy that Hultquist (1958) observed both hypoglycaemia and morphological signs of increased β -cell function in rats injected with iodoacetamide. He suggested that a single injection of iodoacetamide is capable of lowering the blood sugar concentration through an increase of insulin release. Our results *in vitro* lend strong support to this suggestion.

There is less good agreement between the present results and previous observations on the uptake and membranolytic effects of iodoacetate in fish islets.

Whereas the islets of *Cottus scorpius* were found not to concentrate iodoacetate from blood (Havu, 1969), the islets of *ob/ob* mice accumulated iodoacetamide in amounts several times larger than is explicable in terms of a passive distribution in the tissue water. Using slices of toadfish islets, Watkins *et al.* (1970) showed that treatment for 15 min with 0.1 mM-iodoacetate significantly increased the uptake of mannitol, an extracellular-space marker. In contrast, 0.1 mM-iodoacetamide had no measurable effect on the islet-cell permeability to sucrose in the present study. These discrepancies may be accounted for by the fact that fish islets are relatively poor in β -cells (Falkmer, 1961), whereas the islets of *ob/ob* mice consist of more than 90% of β -cells (Hellman, 1965).

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