JCI The Journal of Clinical Investigation

Interacting Effects of Sulfonylureas and Glucose on Cyclic AMP Metabolism and Insulin Release in Pancreatic Islets of the Rat

V. Grill, E. Cerasi

J Clin Invest. 1978;61(5):1346-1354. https://doi.org/10.1172/JCI109052.

Research Article

The effects of tolbutamide and glibenclamide on the metabolism of cyclic AMP were investigated in pancreatic islets of the rat. Changes in cyclic AMP were assessed by measuring [^{3}H]cyclic AMP after labeling of the islets with [2^{3}H]adenine. In the presence of a nonstimulatory concentration of glucose (3.3 mM), both sulfonylureas caused a rapid increase in islet [^{3}H]cyclic AMP, which declined within 5 (tolbutamide) or 10 min (glibenclamide). In the absence of glucose, the glibenclamide effect was shortened, but the initial (1 min) response of [^{3}H]-cyclic AMP was unaffected. Glucose could be substituted with d-glyceraldehyde but not pyruvate for prolongation of the glibenclamide response. The effect of glucose withdrawal on the glibenclamide response was reproduced by the addition of d-mannoheptulose to glucose containing media.

The [^{3}H]cyclic AMP response to glibenclamide was influenced by prior exposure of the islets to glucose, a 30-min preincubation with 27.7 mM glucose, enhancing the response to the sulfonylurea over a subsequent 5-min stimulation period.

Sulfonylureas exerted their effects at low but not at high glucose concentrations, i.e., shifted the glucose dose-response curve to the left both for [^{3}H]cyclic AMP accumulation and insulin release. On the other hand, increasing concentrations of the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine, progressively augmented the effects of the drugs.

Omission of Ca^{++} from the incubation media inhibited both the glucose and the sulfonylurea [^{3}H]-cyclic […]

Interacting Effects of Sulfonylureas and Glucose on Cyclic AMP Metabolism and Insulin Release in Pancreatic Islets of the Rat

V. GRILL and E. CERASI, *Department of Endocrinology, Karolinska Hospital, S-104 01 Stockholm 60, Sweden*

ABSTRACT The effects of tolbutamide and glibenclamide on the metabolism of cyclic AMP were investigated in pancreatic islets of the rat. Changes in cyclic AMP were assessed by measuring [^{3}H]cyclic AMP after labeling of the islets with [2-^{3}H]adenine. In the presence of a nonstimulatory concentration of glucose (3.3 mM), both sulfonylureas caused a rapid increase in islet [^{3}H]cyclic AMP, which declined within 5 (tolbutamide) or 10 min (glibenclamide). In the absence of glucose, the glibenclamide effect was shortened, but the initial (1 min) response of [^{3}H]-cyclic AMP was unaffected. Glucose could be substituted with D-glyceraldehyde but not pyruvate for prolongation of the glibenclamide response. The effect of glucose withdrawal on the glibenclamide response was reproduced by the addition of D-mannoheptulose to glucose containing media.

The [^{3}H]cyclic AMP response to glibenclamide was influenced by prior exposure of the islets to glucose, a 30-min preincubation with 27.7 mM glucose, enhancing the response to the sulfonylurea over a subsequent 5-min stimulation period.

Sulfonylureas exerted their effects at low but not at high glucose concentrations, i.e., shifted the glucose dose-response curve to the left both for [^{3}H]cyclic AMP accumulation and insulin release. On the other hand, increasing concentrations of the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine, progressively augmented the effects of the drugs.

Omission of Ca^{++} from the incubation media inhibited both the glucose and the sulfonylurea [^{3}H]-cyclic AMP and insulin responses. Epinephrine (1 μM) partially inhibited the [^{3}H]cyclic AMP response to both glucose and sulfonylurea, whereas insulin release was completely abolished.

It is concluded that the sulfonylurea effects on islet cyclic AMP are intimately related to those of glucose. It is suggested that sulfonylureas exert a major part of their action by facilitating the effect of glucose on the beta-cell adenylate cyclase; the increased cyclic AMP level, in its turn, enhances the secretion rate of insulin.

INTRODUCTION

The mode of action of sulfonylureas, well-known insulin secreting agents (1), is not well understood. However, in recent years evidence has been accumulating suggesting that the adenylate cyclase-cyclic AMP[1] system of the islets is involved in the transmission of the drug effect. In islet homogenates, tolbutamide stimulates adenylate cyclase to a moderate extent (2) and inhibits phosphodiesterase enzyme activity (3–5). Recently, Charles and co-workers have reported that tolbutamide increases the cyclic AMP level of perifused islets simultaneously with insulin release (6).

Insulin secretion induced by sulfonylureas is dependent upon the prevailing glucose concentration. Thus sulfonylurea induces a shift to the left in the glucose dose-response curve for insulin release (1, 7). The nature of interaction between these drugs and glucose is unknown.

We and others have obtained evidence that glucose increases cyclic AMP concomittantly with insulin secretion, and that this action constitutes an important

Part of this work was previously reported at the symposium "Diabetes Research Today," held in Capri, Italy, 13–15 April 1976, and published in Symposia Medica Hoechst 12, 223–240 (1976), Friedrich-Karl Schattauer-Verlag GmbH, Stuttgart, West Germany.

Dr. Cerasi's present address is the Department of Endocrinology and Metabolism, the Hebrew University Hadassah Medical School, Jerusalem, Israel.

Received for publication 25 July 1977 and in revised form 8 November 1977.

[1] *Abbreviations used in this paper*: cyclic AMP, cyclic adenosine -3′, 5′-monophosphate; IBMX, 3-isobutyl-1-methylxanthine; KHB, Krebs-Henseleit bicarbonate buffer.

factor in the regulation of insulin release (8). Against this background it seemed important to investigate the relationship, if any, between sulfonylurea- and glucose-induced changes in beta-cell cyclic AMP metabolism. To this end, different aspects of a glucose-sulfonylurea interaction were investigated: (*a*), the time-course of sulfonylurea effect in the presence or absence of glucose, (*b*), the effects of preincubation with glucose on the subsequent sulfonylurea response, and (*c*), the effects of sulfonylurea over a range of glucose concentrations. In addition, the effects of different conditions inhibiting insulin release on the glucose- and sulfonylurea-induced responses were compared. An indirect method for assessing changes in cyclic AMP metabolism was utilized, namely the conversion of [^{3}H]ATP to [^{3}H]cyclic AMP in prelabeled islets, a procedure whose validity has been confirmed (9). Two sulfonylureas were chosen: a classical one (tolbutamide), and one representative of the "second generation" of these drugs (glibenclamide).

METHODS

Preparation of islets, labeling with [^{3}H]adenine, and incubation of islets. Male Sprague-Dawley rats weighing 100–150 g were fed ad libitum until decapitation. Pancreatic islets were isolated by the collagenase method of Lacy and Kostianovsky (10). The collagenase employed was from Worthington Biochemical Corp., Freehold, N. J. Krebs Henseleit-bicarbonate buffer (KHB) with 0.2% bovine serum albumin, 10 mM Hepes, and, when not otherwise indicated, 3.3 mM glucose was used throughout the experiments. Islets were pulse labeled with 100 μCi/ml of [2-^{3}H]adenine (25 Ci/mmol, New England Nuclear, Dreieichenhain, W. Germany) during a 60-min preincubation period as previously described (11). Islets were then transferred in batches of 15 either to small incubation tubes with a final incubation volume of 1 ml or, in some experiments, to "baskets." These were prepared by cutting small microcentrifugation plastic tubes with an upper inner diameter of ~4 mm (Beckman Instruments, Munich, W. Germany). The hollow cylinders thus obtained were capped with nylon gauze (70 threads/cm^2 of gauze, 36% open space). The baskets with the islets were placed in small incubation tubes in a final volume of 0.5 ml. In all experiments incubations were carried out at 37°C together with the agents to be tested, and 0.1 mM of 3-isobutyl-1-methylxanthine (IBMX) unless otherwise indicated. This substance, as well as D-glyceraldehyde, was from Aldrich Chemical Co., Milwaukee, Wis. The sulfonylureas (tolbutamide and glibenclamide) were generously supplied by Farbwerke Hoechst A. G., Frankfurt, W. Germany.

Measurement of [^{3}H]cyclic AMP. After removing an aliquot of the incubation medium, 100 μg of cyclic AMP was added and the samples kept in a boiling water bath for 5 min. [^{3}H]cyclic AMP was extracted as previously described (11, 12), and the radioactivity counted by liquid scintillation. Except for the boiling step, [^{3}H]cyclic AMP in incubation media was identically processed. The islet content of [^{3}H]cyclic AMP was thus calculated by subtraction of [^{3}H]cyclic AMP in the medium remaining with the islets.

Insulin assay. Insulin was measured using a charcoal separation method of radioimmunoassay (13). ^{131}I-Labeled pork insulin (obtained from the Radiochemical Centre, Amersham, Buckinghamshire, England) was used. Purified rat insulin (kindly supplied by Dr. J. Schlichtkrull, Novo Research Institute, Bagsvaerd, Denmark) served as standard.

Presentation of results. In view of the considerable inter-experimental variation, quantitative comparisons have been restricted to variables measured within the same experiments. Standard statistical procedures were used, all levels of significance being calculated from paired differences. For clarity, results are expressed, when deemed appropriate, after the subtraction of control values.

RESULTS

Dose dependency of sulfonylurea-induced insulin release. The effects of 50–400 μg/ml of tolbutamide, and 0.5–8.0 μg/ml of glibenclamide were tested together with 3.3 mM glucose in incubations of 30 min (Table I). A maximal response was obtained with 200 μg/ml tolbutamide and 2 μg/ml glibenclamide. These concentrations were chosen for further study. Under our experimental conditions, glibenclamide was a somewhat more potent insulin secretagogue than tolbutamide at their respective maximal doses.

Time course of the sulfonylurea effect on [^{3}H]cyclic AMP in the presence of a nonstimulatory glucose concentration. In the presence of 3.3 mM glucose, both tolbutamide and glibenclamide rapidly increased

TABLE I

Insulin and [^{3}H]Cyclic AMP Responses to Sulfonylurea in 30-min Incubations

Substance tested	[^{3}H]cAMP		Immunoreactive insulin	No. of expt.
μg/ml	*dpm/islet tissue*	*dpm/islet medium*	*μU/islet*	
—	6.9±0.6	1.3±0.1	1.6±0.1	7
Tolbutamide				
50	9.0±1.8	3.8±1.1	4.4±1.4*	
200	6.2±1.0	3.8±0.8*	6.6±1.8*	
400	6.8±1.5	3.6±0.8*	6.6±0.8*	
—	6.6±0.7	1.9±0.6	1.1±0.4	4
Glibenclamide				
0.5	8.4±1.3	6.2±1.7*	6.1±0.9*	
2.0	8.0±0.7*	7.4±1.6*	7.3±0.9*	
8.0	7.5±0.8	5.8±1.5*	6.3±0.9*	
—	6.5±0.4	1.9±0.4	1.5±0.2	7
Tolbutamide				
200	8.1±0.6	4.3±0.8	5.6±0.6	
Glibenclamide				
2.0	6.7±0.9	8.1±0.9‡	8.4±1.1‡	

3.3 mM glucose was present in all incubations.

* $P < 0.05$, or less, significance of difference in comparison with glucose, 3.3 mM.

‡ $P < 0.01$, significance of difference in comparison with tolbutamide, 200 μg/ml.

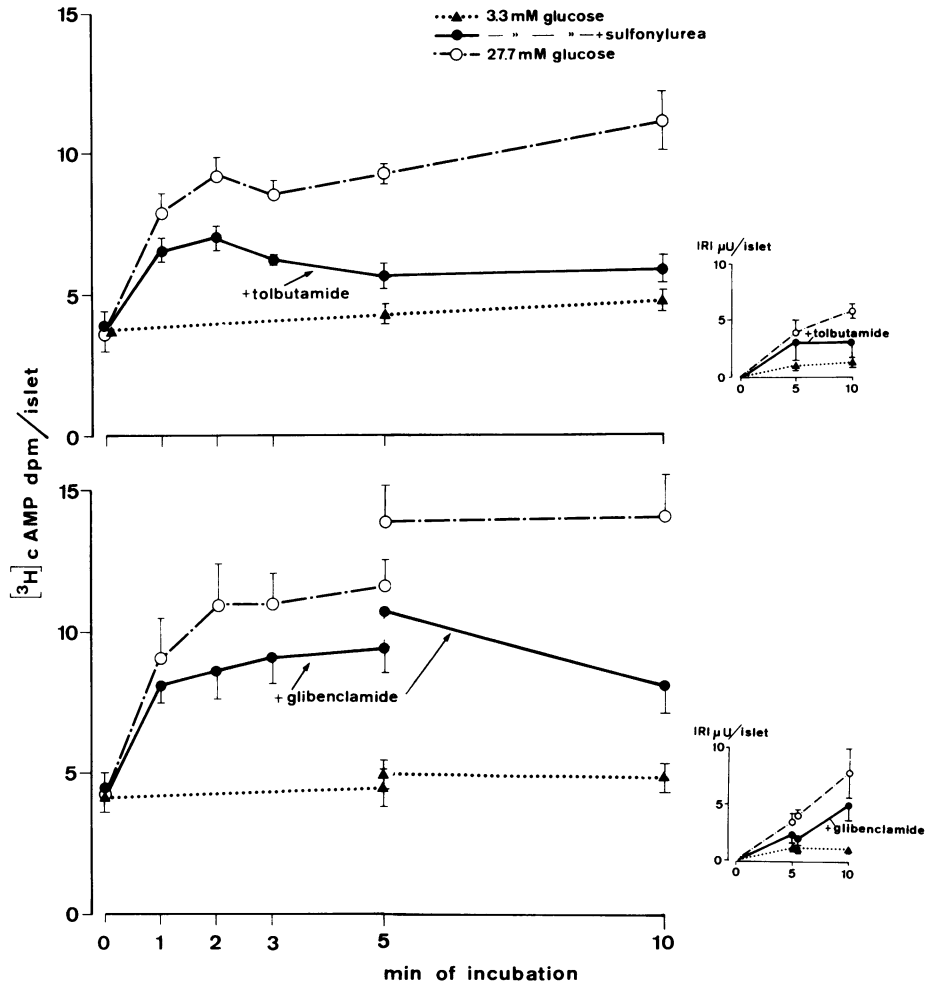

FIGURE 1 Time course of effects of 200 μg/ml tolbutamide or 2 μg/ml glibenclamide on [^{3}H]cyclic AMP accumulation and insulin release in the presence of 3.3 mM glucose. Also the effect of 27.7 mM glucose was tested. Mean±SEM from three separate sets of experiments with tolbutamide, n = 4, with glibenclamide 0–5 min, n = 5, with glibenclamide 5–10 min, n = 6. The release of [^{3}H]cyclic AMP into the incubation medium after 10 min of incubation was 1.7±0.6, 2.1±0.7, and 1.1±0.5 dpm/islet in the experiments with 27.7 mM glucose, glibenclamide, and 3.3 mM glucose, respectively, and 0.7±0.2, 0.6±0.1, and 0.4±0.3 dpm/islet for high glucose, tolbutamide, and low glucose, respectively.

[^{3}H]cyclic AMP accumulation in the islets (Fig. 1). The stimulatory action of tolbutamide seemed to be more short-lived than that of glibenclamide, [^{3}H]-cyclic AMP levels declining by about 50% from the peak value after 5 min in the case of tolbutamide and after 10 min with glibenclamide. In contrast, the glucose effect did not decrease during the 10 min of incubation. In the same experiments, insulin release was augmented both by the drugs and by glucose as measured after 5 and 10 min of incubation.

Efflux of [^{3}H]cyclic AMP into the incubation medium was barely detectable after 10 min of incubation (Fig. 1). After 30 min of incubation, the accumulated efflux of radioactive nucleotide was markedly elevated in the presence of sulfonylureas (Table I). On the other hand, only small and usually insignificant stimulatory effects on islet cyclic AMP remained at this time.

Time course of the sulfonylurea effect in the absence of glucose. When the effects of glibenclamide in the absence or presence of 3.3 mM glucose were compared (Fig. 2), it was apparent that the fall of radioactive nucleotide concentration was much more rapid in the absence than in the presence of glucose, whereas the initial (1 min) rise in [^{3}H]cyclic AMP was not modified. In a parallel fashion, insulin release in response to glibenclamide during 10 min of incubation was decreased in the absence of glucose.

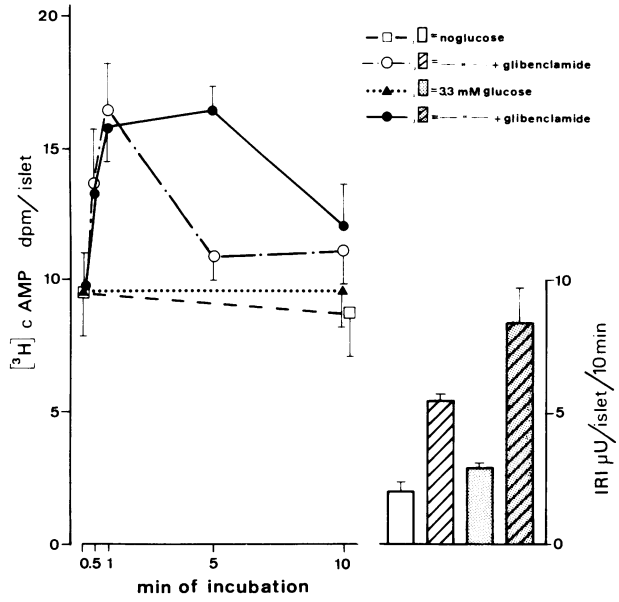

FIGURE 2 Effect of glibenclamide (2 μg/ml) on islet [^{3}H]cyclic AMP and insulin release in the absence or presence of 3.3 mM glucose. Islets were incubated in "baskets" (see Methods). Mean±SEM of one complete set of experiments, $n = 4$. Release of [^{3}H]cyclic AMP was not measured in these experiments.

Effects of D-*mannoheptulose.* To further test the importance of glucose metabolism for sulfonylurea action, the effects of mannoheptulose, an inhibitor of the first step of glycolysis, were tested together with glibenclamide. The concentration of mannoheptulose used completely inhibits the cyclic AMP and insulin responses to 27.7 mM glucose (14). In the absence of glucose, mannoheptulose had no effect on the glibenclamide-induced ^{3}H cyclic AMP response in 1- and 5-min incubations (Table II). In the presence of 3.3 mM glucose, however, although the response after 1 min of incubation was unaffected, the glibenclamide-induced rises in [^{3}H]cyclic AMP and insulin release were inhibited by about 50% after 5 min of incubation.

Effect of substituting 3.3 mM glucose with D-*glyceraldehyde of pyruvate.* In 5-min incubations, a low concentration of the glucose metabolite D-glyceraldehyde (1.5 mM) could at least partly substitute for glucose in prolonging the glibenclamide response, the drug effect on [^{3}H]cyclic AMP being enhanced from 5.1±0.4 to 8.5±1.3 dpm, and insulin release from 0.2±0.1 to 0.4±0.1 μU ($n = 6$, $P < 0.05$ for both parameters, the appropriate control values having been subtracted). Under the same conditions pyruvate (10 mM) was ineffective as a substitute for glucose (results not shown).

TABLE II

Divergent Effects of Mannoheptulose on the Glibenclamide-Induced [^{3}H]Cyclic AMP, and Insulin Responses in the Absence or Presence of 3.3 mM Glucose

Time of incubation	Glucose	Additions	[^{3}H]cAMP	Immunoreactive insulin	No. of expt.
min	*mM*		*dpm/islet*	*μU/islet*	
1	0	Glibenclamide	2.6±0.6	Undetectable	5
	0	Glibenclamide + Mannoheptulose	2.7±0.8		
	3.3	Glibenclamide	3.3±0.7		
	3.3	Glibenclamide + Mannoheptulose	2.7±0.7		
5	0	Glibenclamide	2.1±0.7	0.4±0.1	8
	0	Glibenclamide + Mannoheptulose	2.7±0.6	0.6±0.5	
5	3.3	Glibenclamide	6.6±0.8	0.9±0.2	10
	3.3	Glibenclamide + Mannoheptulose	3.7±0.7*	0.5±0.1*	

The concentrations of glibenclamide and mannoheptulose were 2 μg/ml and 13.8 mM, respectively. Mean±SEM of three separate series of experiments where the appropriate control values have been subtracted.

* $P < 0.05$ or less, significance of difference for the effect of glibenclamide in the absence or presence of mannoheptulose.

Effect of preincubation with 27.7 mM glucose. Because the previous experiments had demonstrated that the sulfonylurea effects are profoundly influenced by the presence of even a small, nonstimulatory concentration of glucose, it was investigated whether preincubation with glucose could modify the subsequent response to sulfonylurea. Islets were incubated for 30 min in 27.7 mM, then for 20 min in 3.3 mM glucose, and then exposed to glibenclamide (together with 3.3 mM glucose). As seen in Fig. 3, the islets which had been preincubated with high glucose responded better to glibenclamide than those exposed to a low glucose concentration, both at an early (1 min) and a later (5 min) time point.

Effect of omission of Ca^{++}. Omission of Ca^{++} from the incubation media profoundly depressed the glibenclamide as well as the glucose-induced [^{3}H]-cyclic AMP and insulin responses in 5-min incubations (Fig. 4).

Effect of epinephrine. In 5 min-incubations epinephrine (1 μM) partially decreased the effect of glibenclamide on [^{3}H]cyclic AMP (tested with 3.3 mM glucose) whereas insulin release was completely inhibited (Fig. 5). The [^{3}H]cyclic AMP response to glibenclamide was totally inhibited upon the further addition of mannoheptulose. In the presence of 27.7 mM glucose, epinephrine reduced the effect of 27.7 mM glucose on [^{3}H]cyclic AMP by 73% whereas insulin release was totally abolished (mean of three experiments, data not shown).

Effect of sulfonylureas together with different concentrations of glucose. The effect of tolbutamide was tested together with a range of glucose concentra-

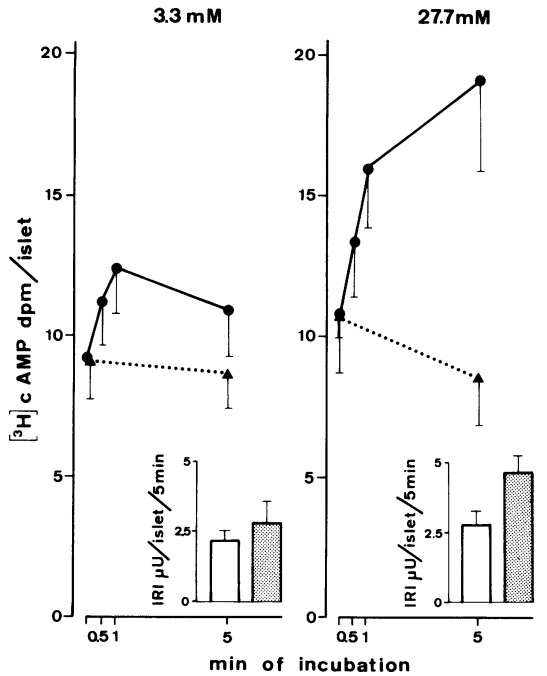

FIGURE 3 Effect of a prior preincubation with 3.3 or 27.7 mM glucose on the glibenclamide-induced [^{3}H]cyclic AMP and insulin responses. Islets were incubated in "baskets" (see Methods) for 30 min either in 3.3 or 27.7 mM of glucose, then for 20 min in 3.3 mM glucose before the final incubations, where 3.3 mM glucose was again present in all incubations. Mean±SEM of one complete set of experiments (n = 5). —●— and ▨ denote incubations with 2 μg/ml of glibenclamide. ···▲··· and □ denote incubations without 2 μg/ml of glibenclamide.

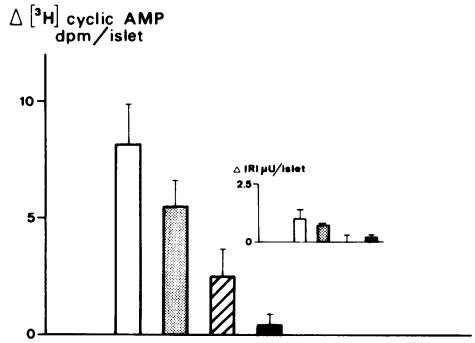

FIGURE 4 Effect of adrenaline (1 μM) on the islet [^{3}H]cyclic AMP accumulation and insulin release induced by glibenclamide (2 μg/ml). 3.3 mM glucose was present in all incubations. Incubation time was 5 min. Mean±SEM of three experiments where the appropriate control values have been subtracted. □ = glibenclamide, ▨ = glibenclamide + mannoheptulose (13.8 mM), ▨ = glibenclamide + adrenaline, ■ = glibenclamide + mannoheptulose + adrenaline.

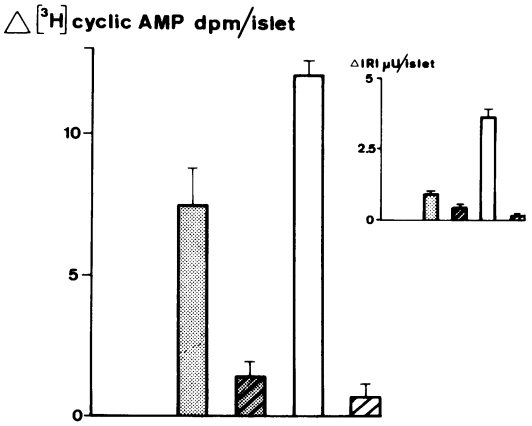

FIGURE 5 Inhibitory effect of omission of Ca^{++} on the glibenclamide- or glucose-induced [^{3}H]cyclic AMP and insulin responses. Incubation time was 5 min. The effects of glibenclamide were tested in the presence of 3.3 mM glucose. In media where $CaCl_2$ was omitted, isoosmolarity was maintained by addition of NaCl. Mean±SEM of three complete experiments where the appropriate control values have been subtracted. ▨ = 2 μg/ml glibenclamide with 2.56 mM Ca^{++}, ▨ = 27.7 mM glucose in the absence of Ca^{++}, □ = 27.7 mM glucose with 2.56 mM Ca^{++}, ■ = glibenclamide in the absence of Ca^{++}.

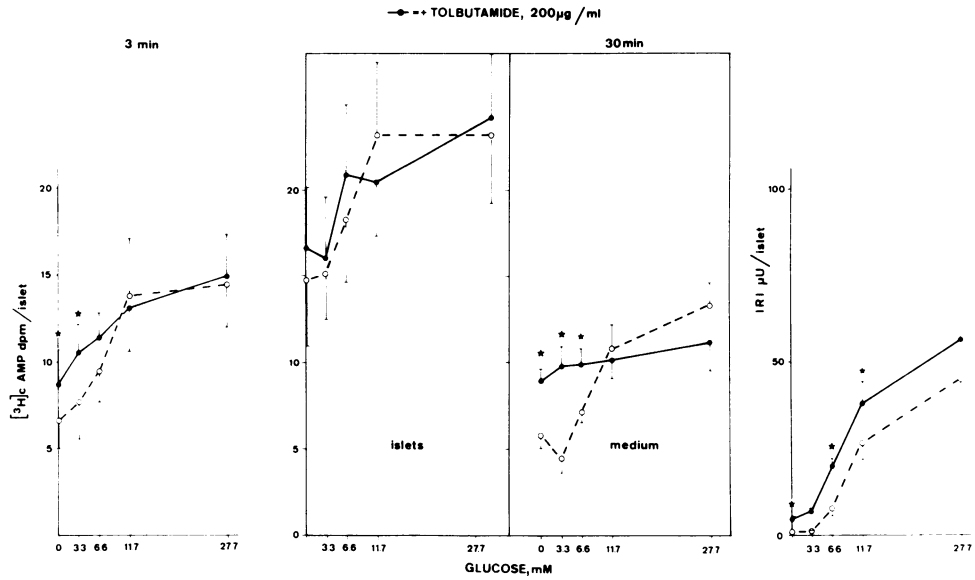

FIGURE 6 Effect of tolbutamide (200 μg/ml) on islet [^{3}H]cyclic AMP accumulation in the presence of 0–27.7 mM glucose after 3 or 30 min of incubation. Mean±SEM of two complete sets of experiments comprising six or seven experiments, respectively. —●—, incubations with, --- ○ ---, without tolbutamide. ★ = $P < 0.05$, significance of difference for incubations with and without tolbutamide. Insulin release was undetectable in the 3-min experiments.

tions in incubations of 3 and 30 min (Fig. 6). A stimulatory effect of tolbutamide on [^{3}H]cyclic AMP, whether on islet (3 min) or on medium (30 min) levels, was seen at low glucose concentrations (0–6.6 mM). Insulin release was stimulated by the tolbutamide in the absence of glucose; this effect was enhanced by the presence of 3.3 or 6.6 mM glucose (P = <0.05) suggesting potentation by low glucose of the sulfonylurea response. In the presence of a high concentration of glucose (27.7 mM), islet [^{3}H]cyclic AMP was unchanged by tolbutamide whereas the efflux of [^{3}H]-cyclic AMP was actually depressed (Fig. 6 and Table III). Insulin release was unaffected by tolbutamide at 27.7 mM glucose (Table I and Fig. 6).

Glibenclamide interacted with glucose in a manner similar to that of tolbutamide (Table III). Glibencla-

TABLE III

Lack of Stimulatory Effect by Sulfonylurea on Insulin and [^{3}H]Cyclic AMP in the Presence of a High Glucose Concentration

Substance tested	Glucose	[^{3}H]cAMP		Immunoreactive insulin	No. of expt.
μg/ml	*mM*	*dpm/islet tissue*	*dpm/islet medium*	*μU/islet*	
—	3.3	9.7±1.5	3.0±0.5	1.4±0.1	16
Tolbutamide 200	3.3	10.8±1.7	6.6±0.8*	6.1±0.5*	
—	27.7	16.7±1.9	13.5±0.9	49.1±4.8	
Tolbutamide 200	27.7	18.3±2.3	10.5±0.8‡	52.2±6.1	
—	3.3	6.4±0.3	1.9±0.3	1.5±0.2	9
Glibenclamide 2.0	3.3	6.4±0.8	8.4±0.8*	8.5±1.1*	
—	27.7	13.3±0.7	13.2±1.4	50.5±7.0	
Glibenclamide 2.0	27.7	14.4±1.4	12.4±1.0	52.7±7.4	

Incubation time was 30 min. Mean±SEM.

* $P < 0.05$, or less, significance of difference for the stimulatory effect of sulfonylureas in comparison with glucose, 3.3 mM.

‡ $P < 0.02$, significance of difference for the inhibitory effect of tolbutamide in comparison with glucose, 27.7 mM.

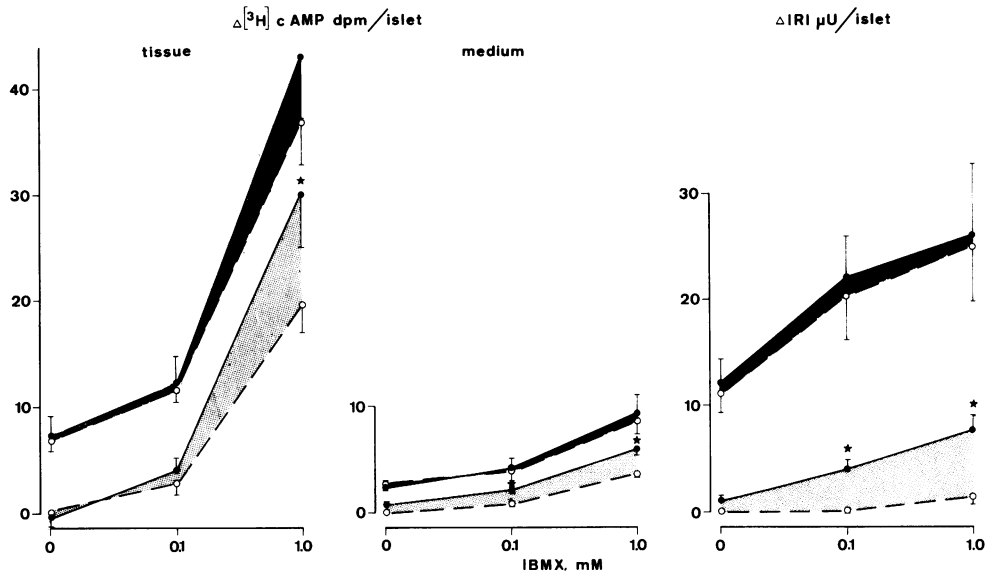

FIGURE 7 Effects of different concentrations of IBMX (0–1 mM) on tolbutamide-induced [^{3}H]cyclic AMP accumulation and insulin release in the presence of 3.3 or 27.7 mM glucose. Incubation time was 15 min. --- ○ ---, control incubations, — ● —, incubations with tolbutamide. Light and dark shaded areas represent the tolbutamide effect in the presence of low or high glucose, respectively. Mean±SEM of six experiments. ★ = $P < 0.05$ or less, significance of difference from the appropriate control.

mide, however, did not inhibit the rise in medium [^{3}H]cyclic AMP evoked by 27.7 mM glucose.

Effects of phosphodiesterase inhibition. To investigate whether the sulfonylurea-induced increase in [^{3}H]cyclic AMP is due to phosphodiesterase inhibition, the effect of tolbutamide was tested together with 0–1 mM of the potent phosphodiesterase inhibitor IBMX (Fig. 7). This substance at 1 mM inhibits the islet enzyme activity almost completely (by 98%);[2] it was therefore assumed that if sulfonylurea and IBMX act on the same sites at the phosphodiesterase enzymes, a sulfonylurea effect would diminish with increasing concentrations of IBMX. As seen in Fig. 7, the tolbutamide effect both on [^{3}H]cyclic AMP and insulin release was, on the contrary, enhanced by increasing concentrations of the phosphodiesterase inhibitor. The effect of glucose (27.7 mM) was magnified in a similar fashion.

DISCUSSION

The present results confirm and extend the observation of Charles and co-workers (6) that sulfonylureas induce a short lasting elevation of the islet cyclic AMP content. Addition of a phosphodiesterase inhibitor in a low concentration in our experiments did not alter the evanescent character of the sulfonylurea action.

The effects of sulfonylureas on cyclic AMP formation on one hand, and on insulin release on the other, seem to follow each other closely. In the study of Charles et al. (6), where islet perifusion permitted moment-to-moment correlations, insulin release was parallel to changes in islet cyclic AMP. Also in our batch-type incubations, sulfonylureas induced changes on islet [^{3}H]cyclic AMP and on insulin secretion which were qualitatively similar. Furthermore, the differences in potency and duration between glibenclamide and tolbutamide, that have been well documented regarding insulin secretion (1), were also found in our system regarding [^{3}H]cyclic AMP formation. All these findings are compatible with the hypothesis that at least part of the secretory effect of sulfonylureas is mediated by their action on the beta-cell adenylate cyclase-cyclic AMP sytem.

To our mind, a most important aspect in the sulfonylurea effect is the interaction that exists between glucose and the drug. Perhaps most striking is the effect of glucose on the timecourse of the sulfonylurea response, where a small, nonstimulatory concentration of glucose (3.3 mM) was sufficient to markedly prolong the stimulating action of glibenclamide on islet [^{3}H]cyclic AMP. Mannoheptulose blocked this time-dependent effect of glucose. These findings are in line with the data of Loubatières-Mariani and co-workers (15), who showed that the later but not the immediate insulin response to tolbutamide was inhibited by mannohep-

[2] Unpublished observations.

tulose. Another aspect of the glucose dependency for the sulfonylurea effect was revealed in experiments where preincubation with glucose augmented not only the later but also the initial [^{3}H]cyclic AMP response to glibenclamide (Fig. 3).[3] Finally the dosekinetics of glucose-induced cyclic AMP accumulation were subject to modification in the presence of the drug: sulfonylureas shifted the glucose dose-response curve to the left. It should be noted that the inability of sulfonylurea to augment the response to high concentrations of glucose is probably not due to limitations in the maximal capacity of the experimental system since both insulin release and [^{3}H]cyclic AMP accumulation in the presence of 27.7 mM glucose could be further augmented by increasing the concentration of IBMX (Fig. 7).

What constitutes the basis for the interaction between glucose and sulfonylurea? No definite answers are available, but several possibilities may be raised. It seems probable that the drugs act at the cell membrane because they do not readily penetrate into the cell (16, 17). One obvious possibility is, then, that sulfonylureas and glucose compete allosterically for a cell membrane receptor, which acts as the signal recognition site that initiates insulin release. The observation that the sulfonylurea but not the glucose effect on cyclic AMP was unaffected by mannoheptulose seems to speak against this explanation. Alternatively, sulfonylureas could facilitate the glucose stimulus for release in an indirect fashion, e.g., by changing ionic fluxes, thus depolarizing the cell membrane (18), or by modifying the membrane in other ways. We have no direct evidence on this point. Finally, and perhaps additionally to the above-mentioned alternatives, glucose may be necessary as a permissive fuel providing agent for sulfonylurea action. Such a role for glucose is suggested by our observation that preincubation with a high glucose concentration augmented the subsequent response to glibenclamide. It is of interest in this respect that islet ATP (19) and glycogen (20) have been shown to be rapidly depleted after stimulations with sulfonylureas. However, this should not necessarily indicate that cyclic AMP stimulation, in the absence of glucose, always decreases rapidly because of lack of substrate. Indeed, another adenylate cyclase stimulator, cholera toxin, stimulates a pronounced and sustained cyclic AMP response in the absence of glucose (21).

Glucose has been shown to stimulate acutely adenylate cyclase from islet homogenates (22), whereas sulfonylureas exert small effects both on the adenylate cyclase (2) and phosphodiesterase enzymes (3–5). Our results show that, in the intact islet, sulfonylureas do not compete with methylxanthines for inhibition of the phosphodiesterase enzymes (Fig. 7). We believe therefore that sulfonylureas and glucose increase cyclic AMP by stimulating islet adenylate cyclase(s) rather than by inhibiting the phosphodiesterase activity.

Tolbutamide (but not glibenclamide) inhibited [^{3}H]-cyclic AMP efflux from the islet. The significance and cause of this observation is unclear. We have recently found that the efflux of cyclic nucleotide from the islet can be modified by agents that affect membrane transport functions (23). It is possible that tolbutamide, especially with prolonged incubation time, influences cell membrane transport processes; data from erythrocytes appear compatible with this idea (24). Although the efflux of nucleotide seems to be a sensitive reflection of the time-integrated cyclic AMP stimulation in many experimental systems (25) including islets, (9, 14), it is obvious that this is not true under all experimental circumstances.

To conclude, our studies suggest that sulfonylureas stimulate insulin release at least partly by stimulating the islet cyclic AMP formation. This effect is strongly modified by the action of glucose. It may therefore be suggested that in vivo the insulinotropic effect of sulfonylureas result from their synergistic action with glucose on the beta-cell adenylate cyclase-cyclic AMP system. The molecular basis for this interaction remains to be clarified.

[3] It should be noted that our observations do not exclude the possibility that preincubation with glucose corrects a "starvation" effect on the sensitivity to glibenclamide exerted by the continuous exposure of the islets to a low glucose concentration. The higher responses seen before and then after an added 50-min incubation with 3.3 mM glucose (compare Fig. 2 and left part of Fig. 3) may be suggestive of such a time-dependent effect by low glucose. However, the considerable inter-experimental variation encountered with the present in vitro system precludes a quantitative comparison between different sets of experiments.

ACKNOWLEDGMENTS

The authors wish to express their sincere thanks to Miss Anita Nylén for her devoted assistance, and to Miss Gudrun Bäcklund and Miss Britt-Marie Witasp for excellent secretarial help.

These studies were supported by grants from the Swedish Medical Research Council (no. B77-19X-04540-03A), the Swedish Diabetes Association, the Åke Wiberg Foundation, Farbwerke Hoechst A. G., and the Svenska Läkarsällskapet.

REFERENCES

1. Loubatières, A. 1972. Therapeutic modification of islet function. *Handb. Physiol.* **1**:(Sect. 7. Endocrinology): 653–664.
2. Kuo, W-N., D. S. Hodgins, and J. F. Kuo. 1973. Adenylate cyclase in islets of Langerhans. Isolation of islets and regulation of adenylate cyclase activity by various hormones and agents. *J. Biol. Chem.* **248**: 2705–2711.
3. Sams, D. J., and W. Montague. 1972. The role of adenosine 3′,5′-monophosphate in the regulation of insulin release. Properties of islet-cell adenosine 3′,5′-cyclic monophosphate phosphodiesterase. *Biochem. J.* **129**: 945–952.

4. Ashcroft, S. J. H., P. J. Randle, and I-B. Täljedal. 1972. Cyclic nucleotide phosphodiesterase activity in normal mouse pancreatic islets. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **20:** 263–266.
5. Bowen, V., and N. R. Lazarus. 1973. Glucose-mediated insulin release: 3′,5′ cAMP phosphodiesterase. *Diabetes.* **22:** 738–743.
6. Charles, M. A., I. Lawecki, A. D. Steiner, and G. M. Grodsky. 1976. Cyclic nucleotides in pancreatic islets. Tolbutamide and arginine-induced insulin release. *Diabetes.* **25:** 256–259.
7. Widström, A., and E. Cerasi. 1973. On the action of tolbutamide in normal man. II. Modulation of glucose-induced insulin release by tolbutamide. *Acta Endocrinol.* **72:** 519–531.
8. Gerich, J. E., M. A. Charles, and G. M. Grodsky. 1976. Regulation of pancreatic insulin and glucagon secretion. *Annu. Rev. Physiol.* **38:** 353–388.
9. Grill, V., E. Borglund, and E. Cerasi. 1977. Cyclic AMP in rat pancreatic islets: evidence for uniform labeling of precursor and product with [^{3}H]adenine. *Biochim. Biophys. Acta.* **499:** 251–258.
10. Lacy, P. E., and M. Kostianovsky. 1967. Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes.* **16:** 35–39.
11. Grill, V., and E. Cerasi. 1974. Stimulation by D-glucose of cyclic adenosine 3′,5′-monophosphate accumulation and insulin release in isolated pancreatic islets of the rat. *J. Biol. Chem.* **249:** 4196–4201.
12. Krishna, G., B. Weiss, and B. B. Brodie. 1968. A simple sensitive method for the assay of adenyl cyclase. *J. Pharmacol. Exp. Ther.* **163:** 379–385.
13. Herbert, V., K-S. Lau, C. W. Gottlieb, and S. J. Bleicher. 1965. Coated charcoal immunoassay of insulin. *J. Clin. Endocr.* **25:** 1375–1384.
14. Grill, V., and E. Cerasi. 1976. Effect of hexoses and mannoheptulose on cyclic AMP accumulation and insulin secretion in rat pancreatic islets. *Biochim. Biophys. Acta.* **437:** 36–50.
15. Loubatières-Mariani, M. M., A. L. Loubatières, and I. Chapal. 1973. Analysis of the stimulating action of tolbutamide on the secretion of insulin using mannoheptulose and diazoxide. *Diabetologia.* **9:** 152–157.
16. Hellman, B., J. Sehlin, and I-B. Täljedal. 1971. The pancreatic β-cell recognition of insulin secretagogues. II. Site of action of tolbutamide. *Biochim. Biophys. Res. Commun.* **45:** 1384–1388.
17. Hellman, B., J. Sehlin, and I-B. Täljedal. 1973. The pancreatic β-cell recognition of insulin secretagogues. IV. Islet uptake of sulfonylurea. *Diabetologia.* **9:** 210–216.
18. Meissner, H. P., and T. J. Atwater. 1976. The kinetics of electrical activity of beta cells in response to a "square wave" stimulation with glucose or glibenclamide. *Horm. Metab. Res.* **8:** 11–16.
19. Hellman, B., L-Å. Idahl, and Å. Danielsson. 1969. Adenosine triphosphate levels of mammalian pancreatic B-cells after stimulation with glucose and hypoglycemic sulfonylureas. *Diabetes.* **18:** 509–516.
20. Hellman, B., and L-Å. Idahl. 1970. On the functional significance of the pancreatic β-cell glycogen. *In* The Structure and Metabolism of the Pancreatic Islets. S. Falkmer, B. Hellman, and I-B. Täljedal, editors. Pergamon Press, Oxford and New York. 253–262.
21. Hellman, B., L-Å. Idahl, Å Lernmark, and I-B. Täljedal. 1974. The pancreatic beta-cell recognition of insulin secretagogues: does cyclic AMP mediate the effect of glucose? *Proc. Natl. Acad. Sci. U. S. A.* **71:** 3405–3409.
22. Capito, K., and C. J. Hedeskov. 1977. Effects of glucose, glucose metabolites and calcium ions on adenylate cyclase activity in homogenates of mouse pancreatic islets. *Biochem. J.* **162:** 569–573.
23. Grill, V., and E. Cerasi. 1977. Cyclic AMP metabolism, and insulin release in pancreatic islets of the rat. Effects of agents which alter microtubular function. *Biochim. Biophys. Acta.* **500:** 385–394.
24. Ariëns, E. J. 1969. Oral antidiabetics. Dose, plasma concentration and effect. *Acta Diabetol Lat.* **1**(Suppl.): 143–176.
25. Exton, J. H., S. B. Lewis, R. J. Ho, G. A. Robinson, and C. R. Park. 1971. The role of cyclic AMP in the interaction of glucagon and insulin in the control of liver metabolism. *Ann. N. Y. Acad. Sci.* **185:** 85–100.