JCI The Journal of Clinical Investigation

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J Clin Invest. 1972;51(2):232-241. https://doi.org/10.1172/JCI106808.

Research Article

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The first of these hypotheses appeared unlikely because db-cAMP and theophylline, in sharp contrast with other agents known to affect glucose metabolism in the beta cell, did not modify glucose-induced calcium uptake by isolated islets incubated at high glucose concentrations. The last hypothesis also appeared unlikely since theophylline did not interfere with the deleterious effect of colchicine on the microtubular system, and since vincristine or colchicine did not differentially affect the respective insulinotropic action of glucose and theophylline. An effect of cAMP upon calcium distribution in the beta cell was suggested by the following findings. Whereas glucose and […]

The Stimulus-Secretion Coupling of Glucose-Induced Insulin Release

VII. A PROPOSED SITE OF ACTION FOR ADENOSINE-3',5'-CYCLIC MONOPHOSPHATE

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ABSTRACT Glucose-induced insulin release is thought to result from the following sequence of events in the beta cell: glucose metabolism leading to the production of a metabolic signal, net calcium uptake by the beta cell in response to the signal, and interaction between calcium and a microtubular-microfilamentous system, leading to emiocytosis of the secretory granules. Dibutyryl-cyclic AMP (db-cAMP) and theophylline are known to potentiate glucose-induced insulin release, their insulinotropic action being most marked at high glucose concentrations. Based on the above mentioned concepts, it was considered in the present experiments that the primary site of action of cAMP in the beta cell could correspond to either a facilitation of glucose metbolism, a modification of calcium distribution, or an interaction with the microtubular-microfilamentous system.

The first of these hypotheses appeared unlikely because db-cAMP and theophylline, in sharp contrast with other agents known to affect glucose metabolism in the beta cell, did not modify glucose-induced calcium uptake by isolated islets incubated at high glucose concentrations. The last hypothesis also appeared unlikely since theophylline did not interfere with the deleterious effect of colchicine on the microtubular system, and since vincristine or colchicine did not differentially affect the respective insulinotropic action of glucose and theophylline. An effect of cAMP upon calcium distribution in the beta cell was suggested by the following findings. Whereas glucose and leucine were unable to promote insulin release in the absence of extracellular calcium, the addition of db-cAMP or theophylline to the calcium-depleted media partially restored the insulinotropic action of glucose and leucine. Moreover, theophylline caused a dramatic increase in ^{45}Ca efflux from perifused islets, even in the absence of glucose. It is concluded that the insulinotropic action of cAMP could be due to a glucose-independent translocation of calcium within the beta cell, from an organelle-bound pool to a cytoplasmic pool of ionized calcium readily available for transport across the cell membrane.

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Received for publication 19 March 1971 and in revised form 30 August 1971.

INTRODUCTION

It was recently postulated that glucose, when metabolized in the beta cell, stimulates calcium uptake, and that calcium in turn triggers the release of insulin by activating a microtubular-microfilamentous system thought to control the migration of secretory granules and their expulsion from the cell in the process of emiocytosis (1, 2). Thus, glucose-induced insulin release is dependent on at least three factors, namely the integrity of glucose metabolism in the beta cell, the presence of a sufficient amount of extracellular calcium, and the functional integrity of the microtubular system. Other insulinotropic agents, such as amino acids and sulfonylureas, also stimulate calcium uptake by the beta cell and also require an intact microtubular system in order to promote insulin release (3, 4).

The initial aim of the present study was to extend these observations to another class of stimulatory agents, which are thought to exert their insulinotropic action by increasing the level of adenosine-3',5'-cyclic mono-

phosphate (cAMP)[1] in the beta cell. Within the framework of the above mentioned model, it was considered that the primary site of action of cAMP could correspond to either a stimulation of glucose metabolism, a modification of calcium transport and distribution in the beta cell, or an effect on the microtubular system. These three hypotheses are here explored. Preliminary results were presented in an abstract form (5).

METHODS

Pancreatic tissue and isolated islets. All experiments were performed with either small pieces of pancreatic tissue (approximately 8–10 mg each) or isolated islets of Langerhans removed from fed female albino rats. The islets were obtained by collagenase digestion of the pancreas (6).

Incubation and perifusion media. All incubations and perifusions were carried out at 37°C in a bicarbonate-buffered medium containing albumin (0.5%, w/v; Fraction V; Sigma Chemical Co., St. Louis, Mo.), and equilibrated against a mixture of O_2 (95%) and CO_2 (5%). This medium has the following ionic composition (in mEq/liter): Na^+ 139, K^+ 5, Ca^{++} 2, Mg^{++} 2, Cl^- 124, and CO_3H^- 24 mEq/liter. A series of experiments was performed with media into which no $CaCl_2$ had been incorporated. Upon assay, the total calcium content of such media averaged 0.22 ± 0.02 mEq/liter ($n = 12$); their concentration of ionized calcium was not determined. Also added to the medium, as required, were glucose, galactose, leucine, and pyruvate (E. Merck AG, Darmstadt, West Germany); theophylline; db-cAMP (N^6, O^2-dibutyryl-adenosine-3′,5′-cyclic monophosphate; Boehringer & Soehne, Mannheim, West Germany); vincristine sulfate (Oncovin; Eli Lilly & Co., Indianapolis, Ind.); colchicine (Sigma Chemical Co., St. Louis, Mo.); diazoxide (Schering-Kahlbaum, Berlin); epinephrine (Adrénaline gauche; Rhône-Poulenc, Paris); *d*-mannoheptulose (Schering, Berlin, Germany); 2,4-dinitrophenol (Merck, Darmstadt, Germany); and tetracaine hydrochloride (Winthrop Laboratories, New York).

Insulin secretion. Insulin release by pancreatic tissue was measured on incubation of small pieces of pancreas, in groups of four pieces each, in media (2.0 ml) containing guinea pig anti-insulin serum (kindly donated by Dr. P. H. Wright, University of Indiana, Indianapolis). The rate of insulin release was deduced from the partial neutralization of the antibodies, and calculated as microunits secreted insulin per milligram of tissue per 90 min incubation (7).

Isolated islets were incubated in groups of eight islets each in media (1.0 ml) containing no anti-insulin serum. The insulin content of these media was assayed by a method previously described (8), rates of secretion being expressed as microunits secreted insulin per islet per 90 min incubation.

Calcium uptake. The method used for measurement of ^{45}Ca uptake by isolated islets has been described in detail elsewhere (9). Briefly, in each experiment, two groups of 150–200 islets each were incubated for 90 min in media (1.0 ml) containing $^{45}CaCl_2$ (12.5 μCi/ml; New England Nuclear Corp., Boston, Mass.). After incubation, the islets were submitted to repeated washes in order to remove extracellular ^{45}Ca, and transferred in groups of five islets each in counting vials for measurement of their radioactive content by liquid scintillation. Calcium uptake was expressed either as picograms per islet per 90 min, or as per cent of the control value found in a paired group of islets.

Perifusion of islets. The control and experimental media used for perifusion were kept at 37°C and continuously mixed with O_2 (95%) and CO_2 (5%). The reservoirs were connected to a small perifusion chamber (volume, 0.25 ml; kindly provided by Dr. I. A. Mirsky, University of Pittsburgh, Pittsburgh, Pa.) through a Y-shaped valve. The perifusate was delivered at a constant rate (0.83–0.88 ml/min) by means of a finger pump.

For measurement of insulin release, a group of 200 islets was placed in the chamber and perifused at 37°C for 2 hr. The control and experimental media contained glucose at 1.7 and 16.7 mM concentrations, respectively. The effluent was continuously collected over periods of 1–4 min each, its insulin content being assayed in 0.5 ml portion parts by the method of Wright, Malaisse, and Reynolds (8). The results are expressed in Fig. 2 as microunits secreted insulin per islet per minute.

For measurement of ^{45}Ca efflux, groups of 200 islets each were preincubated for 60 min at 37°C in media (1.0 ml) containing glucose (16.7 mM) and ^{45}Ca (50 μCi/ml). After preincubation, the islets were submitted to repeated washes in order to remove extracellular ^{45}Ca; transferred to the perifusion chamber; and perifused at 37°C for 80 min. During the first 45 min, the perifusate was derived from a first reservoir and contained no theophylline. At the 45th min, the perifusate was derived from the second reservoir which contained either the same medium as that used during the first period (control experiments), or a medium enriched with theophylline (3.2 mM). In these experiments, all media used for perifusion were glucose-free. The effluent was continuously collected in counting vials, over successive periods of 1 min each. To each vial, 10 ml of scintillation fluid (Insta-Gel; Packard Instrument Co., Inc., Downers Grove, Ill.) was added. In each experiment, ^{45}Ca efflux (cpm/min) was expressed as per cent of the value found at the 45th min of perifusion.

RESULTS

Effects of theophylline and db-cAMP on insulin secretion and calcium uptake by isolated islets. Table I illustrates the effect of glucose, theophylline, and db-cAMP on insulin secretion by isolated islets. Insulin release increased as the glucose concentration of the incubation medium was raised from 5.6 to 16.7 mM. Theophylline and db-cAMP failed to affect insulin secretion in the absence of glucose or at low glucose levels, but markedly enhanced glucose-induced insulin release at high glucose concentrations. Table II illustrates the effect of the same agents on calcium uptake by the isolated islets. Glucose stimulated calcium uptake. Both theophylline and db-cAMP inhibited calcium uptake in the absence of glucose, slightly enhanced calcium uptake at a low glucose concentration (5.6 mM), and failed to significantly affect calcium uptake at higher glucose levels. These data, illustrated in Fig. 1, clearly indicate that the insulinotropic action of theophylline and db-cAMP observed at high glucose levels cannot

[1] *Abbreviations used in this paper:* cAMP, adenosine-3′,5′-cyclic monophosphate; db-cAMP, dibutyryl-cyclic adenosine monophosphate; TSH, thyroid-stimulating hormone.

TABLE I

Effect of Glucose, Theophylline, and db-cAMP upon Insulin Secretion by Isolated Islets

Glucose	Control output*	Drug-induced change‡	
		Theophylline (1.4 mM)	db-cAMP (0.2 to 0.4 mM)
mM	*μU/islet per 90 min*	*+ or − μU/islet per 90 min*	
Nil	10.8 ±3.0 (38)	−1.7 ±1.4 (8)	+3.0 ±1.4 (10)
1.7	13.1 ±2.8 (32)	+3.2 ±2.8 (16)	
2.8	13.7 ±2.2 (26)	−1.8 ±2.4 (16)	
4.2	15.5 ±1.7 (16)	+22.5 ±2.9§ (14)	
5.6	29.7 ±1.8 (42)	+32.8 ±4.9§ (12)	+18.5 ±5.0§ (16)
8.3	91.1 ±7.5 (17)	+146.5 ±7.4§ (8)	+120.8 ±10.5§ (8)
16.7	236.3 ±6.6 (93)	+253.5 ±14.2§ (13)	+212.4 ±35.7§ (18)

* Mean values (±SEM) for glucose-induced insulin release; number of observations in parentheses.

‡ Effects of theophylline or db-cAMP are shown as mean differences (±SEM) from control values obtained in equal numbers and within the same experiment(s); also shown are the number of observations (in parentheses).

§ The statistical significance ($P < 0.001$) of the observed changes is shown.

be accounted for by an additional increment in the net accumulation of calcium induced by glucose.

Induction of insulin secretion in calcium-depleted media. When no calcium was incorporated into the incubation medium, both glucose and leucine failed to stimulate insulin release from isolated islets of Langerhans (Table III, lines 2 and 3). Theophylline, db-cAMP, or the combination of these two agents also failed to significantly affect insulin release in the calcium-depleted media (Table III, lines 4–6). However, in the simultaneous presence of glucose and db-cAMP, a marked stimulation of insulin release was noticed (Table III, line 7). The secretion rate was even higher with the combination of glucose and theophylline (Table III, line 8). The rate of insulin release observed under the latter experimental condition (85 ±4 μU/islet per 90 min) remained, however, much lower than that induced by glucose alone at a normal calcium level (236 ±7 μU/islet per 90 min; see Table I). The insulinotropic action of the combination of glucose and theophylline was abolished by mannoheptulose, dinitrophenol, epinephrine, diazoxide, or tetracaine (Table III, lines 9–13). Other metabolic substrates than glucose were also tested. A significant enhancement of insulin release was induced by theophylline in the presence of leucine (Table III, line 15 vs. 3); whereas, neither galactose nor pyruvate in association with theophylline were able to significantly enhance the low rate of insulin release observed in the presence of theophylline alone (Table III, lines 14 and 16 vs. 5).

TABLE II

Effect of Glucose, Theophylline, and db-cAMP upon ^{45}Ca Uptake by Isolated Islets

Glucose	Control uptake*	Experimental value‡	
		Theophylline (1.4 mM)	db-cAMP (1.0 mM)
mM	*pg/islet per 90 min*	*% of Control*	
Nil	66 ±5 (39)	73.5 ±4.6§ (26)	72.5 ±4.9§ (13)
5.6	93 ±6 (51)	145.5 ±9.9§ (26)	124.8 ±8.8‖ (25)
8.3	173 ±8 (58)	107.5 ±4.3 (26)	103.6 ±4.4 (22)
16.7	249 ±12 (62)	96.3 ±2.8 (32)	103.9 ±4.4 (26)

* Mean absolute values (±SEM) for glucose-induced ^{45}C uptake; number of observations in parentheses.

‡ Experimental values obtained in the simultaneous presence of glucose and either theophylline or db-cAMP are quoted as per cent of their paired control value; also shown are the number of individual observations (in parentheses).

§ The statistical significance ($P < 0.001$) of the observed changes is shown.

‖ $P < 0.01$.

The present experiments thus confirm that glucose and leucine are unable to stimulate insulin release in the absence of sufficient extracellular calcium. They also suggest that db-cAMP or theophylline modify the internal milieu of the beta cell in such a way that the normal supply of extracellular calcium is no longer necessary for glucose or leucine to stimulate insulin secretion.

Insulin release and calcium efflux from perifused islets. In view of the preceding findings, a possible effect of theophylline on the intracellular distribution of calcium was considered. In order to test this hypothesis, we decided to examine the influence of theophylline upon ^{45}Ca efflux from perifused islets.

Fig. 2 illustrates the secretory response of the perifused islets to glucose. When the glucose concentration of the perifusate was raised from 1.7 to 16.7 mM, a brisk increase in insulin output was noticed. This early response was followed by a progressive buildup of the secretory rate. After exposure for 30–40 min to the high glucose concentration, insulin output reached a plateau corresponding to a mean secretory rate of 2 μU/islet per minute. This value is of the same order of magnitude as that observed on prolonged incubation of isolated islets (see Table I). A rapid and marked decrease in insulin output occurred when the glucose concentration of the perifusate was lowered. This secretory pattern was taken as an indication of the functional integrity of the perifused islets.

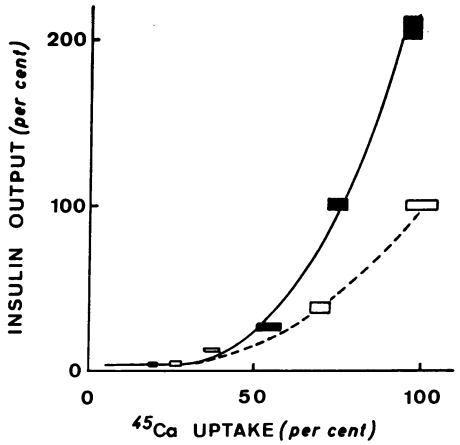

FIGURE 1 Relationship between ^{45}Ca uptake and insulin output by isolated islets. Each rectangle represents the mean (±SEM) for ^{45}Ca uptake and insulin output, both parameters being expressed as per cent of the value found at high glucose concentration (16.7 mM). Open rectangles refer to increasing glucose concentrations (Nil, 5.6, 8.3, and 16.7 mM) in the absence of theophylline; closed rectangles refer to the same glucose concentrations in the presence of theophylline (1.4 mM).

When the islets preincubated in the presence of ^{45}Ca were perifused with a glucose-free solution, the efflux of ^{45}Ca rapidly decreased during the first ½ hr of perifusion (data not shown). Thereafter, the decrease in ^{45}Ca efflux

TABLE III

Insulin Secretion by Islets Incubated in Calcium-Depleted Media

Line No.	Substrate	Potentiator	Inhibitor	Insulin output*	P‡
	mM	mM	mM	μU/islet per 90 min	
1				9.8 ±1.1(103)	
2	Glucose (16.7)			11.9 ±1.2 (82)	vs. 1, NS
3	Leucine (20.0)			8.5 ±3.0 (9)	vs. 1, NS
4		db-cAMP (1.0)		4.3 ±1.3 (10)	vs. 1, NS
5		Theophylline (1.4)		12.5 ±1.2 (22)	vs. 1, NS
6		db-cAMP (1.0) and theophylline (1.4)		8.1 ±1.2 (10)	vs. 1, NS
7	Glucose (16.7)	db-cAMP (1.0)		27.6 ±2.0 (34)	vs. 2, <0.001
8	Glucose (16.7)	Theophylline (1.4)		85.4 ±3.5 (40)	vs. 2, <0.001
9	Glucose (16.7)	Theophylline (1.4)	Mannoheptulose (12.0)	6.3 ±1.2 (10)	vs. 8, <0.001
10	Glucose (16.7)	Theophylline (1.4)	Dinitrophenol (0.4)	8.6 ±1.8 (10)	vs. 8, <0.001
11	Glucose (16.7)	Theophylline (1.4)	Epinephrine (0.01)	7.3 ±1.1 (10)	vs. 8, <0.001
12	Glucose (16.7)	Theophylline (1.4)	Diazoxide (0.43)	2.3 ±1.4 (10)	vs. 8, <0.001
13	Glucose (16.7)	Theophylline (1.4)	Tetracaine (0.5)	17.3 ±1.9 (10)	vs. 8, <0.001
14	Galactose (16.7)	Theophylline (1.4)		14.9 ±2.7 (10)	vs. 5, NS
15	Leucine (20.0)	Theophylline (1.4)		28.0 ±2.5 (10)	vs. 3, <0.001
16	Pyruvate (20.0)	Theophylline (1.4)		17.7 ±3.3 (10)	vs. 5, NS

* All experiments were performed with media into which no calcium had been incorporated; mean values (±SEM) for insulin output are shown together with the number of observations (in parentheses).

‡ The statistical significance of differences (NS, not significant) always refer to a group comparison performed on equal numbers of data obtained within the same experiment(s).

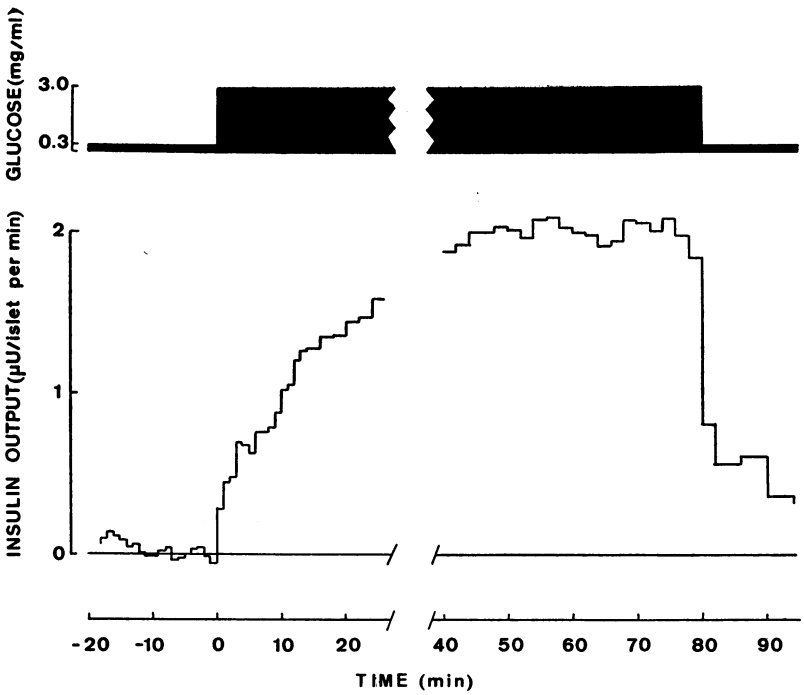

FIGURE 2 Insulin output by perifused islets. Mean value for, respectively six (left part) and four (right part) experiments. In each experiment, a group of 200 islets was placed in the perifusion chamber at −25 min. The glucose concentration of the perifusate is shown in the upper part of the figure.

occurred at a slower rate, the mean value progressively decreasing between the 32nd and 81st min from 119 ±3 to 70 ±3% of the reference value observed at the 45th min (Fig. 3). The addition of theophylline to the perifusate provoked a marked and sustained increase in ^{45}Ca efflux. The rise in ^{45}Ca efflux was already detectable within the 1st min of exposure to theophylline. At its maximal value, ^{45}Ca efflux in the presence of theophylline averaged twice the control value found in the absence of methylxanthine ($P < 0.03$).

Effects of theophylline, vincristine, and colchicine upon insulin release. In the experiments summarized in Table IV, pieces of pancreatic tissue were preincubated for 90 min in media containing glucose (5.6 mM) and, as required, vincristine, colchicine, and theophylline. After this preincubation, the pieces were incubated for 90 min in a second medium containing a higher amount of glucose (16.7 mM) with or without theophylline. Insulin secretion was measured only during the final incubation period.

When the pieces of pancreatic tissue were preincubated in the presence of either vincristine or colchicine and, thereafter, transferred to a new medium containing no inhibitor, their secretory response to glucose was significantly impaired (Table IV, lines 3 and 5 vs. line 1). The addition of theophylline to the final incubation medium significantly enhanced glucose-induced insulin secretion, whether preincubation had been carired out in the absence or presence of a mitotic-spindle inhibitor (Table IV, lines 2 vs. 1, 4 vs. 3, and 6 vs. 5). However, after preincubation with either vincristine or colchicine, the rate of insulin secretion induced by the combination of glucose and theophylline was significantly lower than that observed with the same combination after preincubation in the absence of any inhibitor (Table IV, lines 4 and 6 vs. line 2). Prior exposure to vincristine inhibited to a comparable degree the secretory responses to glucose alone (47.4% inhibition) and to the combination of glucose and theophylline (42.5% inhibition). Prior exposure to colchicine also reduced to a same extent the secretory responses to glucose alone (39.6% inhibition) and to the combined stimuli (34.0% inhibition). After preincubation in the simultaneous presence of colchicine and theophylline, the secretory rate induced by glucose was not significantly different from that observed after exposure to colchicine alone (Table IV, lines 5 and 7).

These data suggest that the alteration of the microtubular system induced by colchicine or vincristine does not differentially affect the respective insulinotropic ac-

tion of glucose and theophylline; and that theophylline itself does not interfere with the deleterious effect of colchicine on the microtubular system.

DISCUSSION

It is now firmly established that the intracellular accumulation of cAMP stimulates insulin release from the pancreatic beta cell. Samols, Marri, and Marks (10) were the first to demonstrate that glucagon, an activator of adenylcyclase, promotes insulin secretion. Turtle and Kipnis (11) observed that glucagon indeed increases the level of cAMP in isolated islets. Numerous reports (12–19) have also documented the insulinotropic action of various agents thought either to activate adenylcyclase (isoproterenol, glucagon, enteroglucagon, ACTH, TSH) or to inhibit phosphodiesterase (theophylline, caffeine); cAMP and db-cAMP were also shown to cause insulin release (16, 20, 21).

The present study aims at locating the primary site of action of cAMP in the beta cell. Three hypotheses are considered, namely a stimulation of glucose metabolism, an effect of cAMP upon calcium metabolism, and an interaction between the nucleotide and the microtubular protein.

Cyclic AMP as a modulator of glucose metabolism. Samols, Marri, and Marks (22) were also the first to suggest that cAMP exerts its insulinotropic action by facilitating glucose metabolism in the beta cell. This concept appeared to be substantiated when it was shown that all agents thought to increase the cellular level of cAMP, and even cAMP itself or db-cAMP, are only able to cause sustained hypersecretion of insulin in vitro if the beta cell is provided with a sufficient supply of either extracellular or intracellular glucose (15, 20, 21). Moreover, whenever the metabolism of glucose in the beta cell is inhibited by such agents as mannoheptulose and 2-deoxyglucose, the enhancing effect of glucagon or theophylline upon glucose-induced insulin release is also suppressed (15, 20).

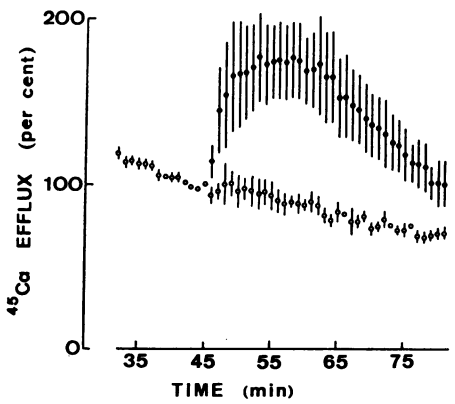

FIGURE 3 ^{45}Ca efflux from perifused islets. In each experiment, 200 islets were preincubated in the presence of ^{45}Ca, and placed in the perifusion chamber at time zero. The perifusate was a glucose-free bicarbonate-buffered medium. Open circles refer to data obtained in the absence of theophyyline, and closed circles to data obtained in the presence of theophylline (3.2 mM). In each experiment, ^{45}Ca efflux (cpm/min) was expressed as per cent of the value found at the 45th min. Mean values (±SEM) refer to seven experiments up to the 45th min; and, respectively, to four (closed circles), and three (open circles) experiments thereafter.

As soon as these data were published, it was observed that glucagon and theophylline do not only potentiate glucose-induced insulin release, but also enhance the insulinotropic action of certain amino acids, such as leucine and glycine (15, 23). Since the latter results were recorded in the absence of glucose, they did not support

TABLE IV

Effect of Theophylline and Mitotic-Spindle Inhibitors upon Insuline Secretion by Incubated Pieces of Pancreatic Tissue

Line No.	Preincubation* (Glucose, 5.6 mM) Added drug, mM	Incubation* (Glucose, 16.7 mM) Added drug, mM	Insulin output‡	P
1	Nil	Nil	100.0 ±10.0 (44)	
2	Nil	Theophylline, 1.4	167.8 ±16.9 (26)	vs. 1, <0.01
3	Vincristine, 0.03	Nil	52.6 ±10.2 (13)	vs. 1, <0.01
4	Vincristine, 0.03	Theophylline, 1.4	96.6 ±15.0 (13)	vs. 2, <0.01; vs. 3, <0.05
5	Colchicine, 1.0	Nil	60.4 ±12.7 (28)	vs. 1, <0.05
6	Colchicine, 1.0	Theophylline, 1.4	110.9 ±12.9 (13)	vs. 2, <0.01; vs. 5, <0.02
7	Colchicine, 1.0 and theophylline, 1.4	Nil	50.5 ±11.1 (28)	vs. 1, <0.01; vs. 5, NS

* Both the preincubation and incubation were carried out for 90 min at 37°C.

‡ Mean values (±SEM) for insulin output during incubation are expressed as per cent of the mean control rate of secretion observed within the same experiment; also shown are the number of observations (in parentheses) and the statistical significance (*P*) of differences between mean values (NS, not significant).

the concept of a specific effect of cAMP on glucose metabolism (15).

The measurement of calcium uptake by isolated islets offered a new opportunity to test the possible effect of cAMP on glucose metabolism. Indeed, we have shown elsewhere that calcium uptake is regulated by the amount of glucose available to and metabolized in the beta cell (9). Thus, glucose stimulates the accumulation of calcium by inhibiting calcium efflux from the beta cell (24); and inhibitors of glucose metabolism, such as mannoheptulose and 2-deoxyglucose, inhibit the stimulant action of glucose upon calcium uptake (9). Therefore, if cAMP were to enhance glucose-induced insulin secretion by accelerating glucose metabolism in the beta cell, it should theoretically also enhance glucose-induced calcium uptake.

In the present experiments, a modest but significant potentiation of glucose-induced calcium uptake by either theophylline or db-cAMP was only observed at a 5.6 mM glucose concentration which is close to the threshold value for the stimulant action of glucose upon both calcium uptake (9) and insulin release (17). Therefore, it is possible that, at this particular glucose level, cAMP indeed accelerates some rate-limiting step of glucose metabolism, as suggested elsewhere (20). However, at higher glucose concentrations, no significant effect of either theophylline or db-cAMP upon glucose-induced calcium uptake could be detected (Table II), suggesting that the marked insulinotropic action of these agents at high glucose levels is not due to a facilitation of glucose metabolism in the beta cell.

The available biochemical data also do not suggest that cAMP has any major effect on glucose metabolism in the beta cell. Thus, Hellerström and Gunnarsson (25) have shown that, whereas glucose (16.7 mM) increases by about 50% the endogenous respiration of isolated islets, both glucagon and db-cAMP slightly depress the oxygen uptake of islets incubated in the presence of glucose (11.1 mM). Ashcroft and Randle (26, 27) reported that, in the presence of glucose (7.4 mM), glucagon produces a slight fall in the rate of glucose oxidation by mouse islets and does not change their glucose-6-phosphate concentration. Finally, according to Hellman and Idahl (28), glucose increases the ATP content of isolated islets, whereas the level of ATP remains unaltered when the beta cell is stimulated by db-cAMP.

Cyclic AMP as a modulator of calcium metabolism. Rasmussen and Tenenhouse (29) have suggested that the effects of cAMP in a variety of systems are due mainly to a modification of calcium metabolism at the cellular level. Thus, the insulinotropic action of cAMP could be due to an altered handling of calcium by the beta cell. If insulin secretion is controlled by the cellular concentration of calcium, as suggested elsewhere (9), cAMP could enhance insulin release by provoking a translocation of calcium within the beta cell, from a pool stored in some organelles to a cytoplasmic pool.

Two arguments can be found in favor of the latter hypothesis. Firstly, theophylline causes a rapid and sustained increase in ^{45}Ca efflux from prelabeled islets. This finding illustrated in Fig. 3 is reminiscent of the rapid increase in ^{45}Ca efflux induced by glucagon or cAMP in the perfused liver (30). It suggests a sudden enrichment of a pool of ionized calcium readily available for transport across the cell membrane. Secondly, db-cAMP and theophylline are able to compensate, to a certain extent, for a lack of extracellular calcium. Thus, in calcium-depleted media, the amount of extracellular calcium available to the beta cell is obviously insufficient to allow glucose or leucine to exert their normal insulinotropic action; whereas, in the presence of db-cAMP or theophylline, the insulinotropic effect of these metabolic substrates is partially restored (Table III).

The concept of a cAMP-induced redistribution of calcium would be consistent with the effects of db-cAMP and methylxanthines in adipose tissue where these agents are also thought to cause a shift of calcium from a bound to a free pool (31). The recent demonstration of adenylcyclase activity in cardiac sarcoplasmic reticulum (32) suggests that the well-known calcium-translocating effect of caffeine in muscle might also be mediated through cAMP accumulation. We have underlined elsewhere the analogy between the processes of stimulus-secretion coupling in the pancreatic beta cell and excitation-contraction coupling in muscle (33). The present data apparently extend this analogy to the calcium-translocating effect of cAMP which results respectively in enhanced insulin release and muscular contracture (29).

Glucose-independency of cAMP-induced calcium translocation. The increase in ^{45}Ca efflux induced by theophylline is apparently independent of glucose, since it was observed in a glucose-free medium. Such effect of theophylline contrasts with that of glucose which was recently shown to inhibit ^{45}Ca efflux from the isolated islets (24). Because glucose and cAMP apparently influence calcium metabolism in the beta cell through different mechanisms, it is unlikely that glucose exerts its own insulinotropic effect solely by provoking an intracellular accumulation of cAMP, as suggested by some authors (34). Other arguments against such idea are the inability of db-cAMP per se to mimic the sustained stimulant action of glucose on insulin release (21), the maintenance of a significant insulinotropic action of glucose despite full activation of phosphodiesterase by imidazole (35), and the failure of glucose to affect the cellular level of cAMP in isolated islets (36, 37).

Substrate-dependency of cAMP-induced insulin re-

lease. If cAMP translocates calcium within the beta cell and increases the concentration of this cation in the cytosol, and furthermore if the level of calcium in the cytosol regulates insulin secretion, it is apparently surprising that the nucleotide is unable to cause sustained insulin release in the absence of glucose (20, 21). This glucose-dependency probably does not correspond to an energy requirement of the beta cell. Thus, pyruvate, which is known to increase ATP level in the beta cell (38), cannot replace glucose as a substrate for the insulinotropic action of cAMP (Table III). Moreover, leucine, which is a suitable substrate for such action, has apparently not to be metabolized in order to stimulate insulin release (39).

It could be speculated that the cAMP-induced translocation of calcium is only able to promote sustained secretion of insulin when the beta cell is simultaneously exposed to a suitable insulinotropic substrate (e.g. glucose or leucine) which itself favors calcium accumulation in the cytosol by inhibiting calcium efflux. That glucose indeed acts in such a manner has been recently demonstrated in this laboratory (24).

This hypothesis would account for all available experimental data. Firstly, it would explain why the accumulation of cAMP does not cause insulin secretion in the absence of either an extracellular or intracellular source of insulinotropic substrate (15, 20). Actually, in the absence of glucose, the cAMP-induced translocation of calcium and subsequent efflux of calcium from the beta cell should reduce the total load of cellular calcium. That this is indeed the case is suggested by the significant lowering of calcium uptake induced by either theophylline or db-cAMP in the absence of glucose (Table II). Secondly, the proposed model would explain why the enhancing action of cAMP upon insulin secretion is more marked at high than at low glucose levels (20). Indeed, the insulinic response evoked by the translocation of a fixed amount of calcium will depend on the actual balance between calcium influx and efflux, i.e. on the ability of the beta cell to retain intracellularly part or all of the translocated calcium. In support of this concept, we have recently observed that the increase in ^{45}Ca efflux from isolated islets induced by theophylline is much less pronounced in the presence than in the absence of glucose (unpublished observations). Thirdly, the model implies that any agent which suppresses the insulinotropic action of glucose should also abolish the enhancing action of glucagon or theophylline. That this is indeed the case has already been documented (15, 20), and is here confirmed (Table III) with mannoheptulose, dinitrophenol, diazoxide, epinephrine, and tetracaine.

Teleologically, the proposed model offers certain advantages. For instance, the substrate dependency of cAMP-induced insulin release is likely to protect the organism against excessive insulin secretion which could otherwise result from the release of pancreatic glucagon in glucopenic situations (40).

Cyclic AMP as a modulator of tubulin metabolism. Lacy, and Lacy, Howell, Young, and Fink (2, 41) have suggested that the microtubular-microfilamentous system of the beta cell plays a role in the insulin-secretory process. Further investigations have confirmed that the integrity of the microtubular system is required in order for glucose and other agents to exert their stimulant action upon insulin release (33, 42). Because the microtubular protein might serve as a substrate for a cAMP-dependent protein kinase (43), the insulinotropic effect of the nucleotide could conceivably be due to such interaction between cAMP and the tubulin molecule. Moreover, it has also been reported that cAMP interferes with the binding of colchicine to the microtubular protein (44). However, the data summarized in Table IV indicate that theophylline does not interfere with the deleterious effect of colchicine, and that the impairment of the microtubular system induced by mitotic-spindle inhibitors does not preferentially affect the respective insulinotropic actions of either glucose or theophylline. These data do not suggest, although they do not entirely rule out, a primary effect of cAMP on the microtubular system. At least, the present findings are compatible with the concept that the participation of the microtubular-microfilamentous system represents an obligatory and late event in the secretory sequence, whatever agent is used to promote insulin release (3).

Conclusions. The present experimental data were taken as an indication that cAMP provokes a redistribution of calcium in the beta cell. This effect of cAMP is apparently independent of the presence of glucose. It might thus represent a fundamental action of cAMP likely to occur in other systems. As such, it could account for the ubiquitous stimulatory effect of cAMP upon calcium-dependent secretory processes (45).

ACKNOWLEDGMENTS

This work was supported in part by grants from the Fonds National de la Recherche Scientifique and Fonds de la Recherche Scientifique Médicale, Brussels, Belgium.

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