Relation of Insulin Release to Cyclic AMP Content in Rat Pancreatic Islets Maintained in Tissue Culture

Alexander Rabinovitch, M.D., Guy S. Cuendet, Ph.D., Geoffrey W. G. Sharp, Ph.D., Albert E. Renold, M.D., and Daniel H. Mintz, M.D., Geneva, Switzerland and Miami, Florida

SUMMARY

This study was designed to elucidate the role of adenosine 3′, 5′-monophosphate (cAMP) in the decreased glucose-induced insulin release observed in cultured pancreatic islets. Freshly collagenase-isolated rat islets and islets that had been maintained in tissue culture medium for two to 140 hours (six days) were incubated for one hour with ^{3}H-2-adenine, and insulin release and islet ^{3}H-cAMP accumulation were measured in subsequent short-term (3 to 60 minutes) incubations.

There was a progressive decrease in the stimulatory effect of 20 mM glucose on insulin release in islets that had been cultured for 2, 4, and 20 hours, and, after 20 to 140 hours of culture, glucose-induced insulin release was about one-third that in freshly isolated islets. Similarly, 20 mM glucose stimulated a threefold increase in ^{3}H-cAMP content in fresh islets, but did not increase ^{3}H-cAMP in islets cultured for four to 140 hours. Basal insulin release and islet ^{3}H-cAMP levels were two- to threefold higher in islets cultured for 44 hours in 16.7 mM glucose rather than in 5.6 mM glucose. However, the sensitivity and capacity of glucose-induced insulin release were less in islets cultured in medium with either 5.6 or 16.7 mM glucose than in freshly isolated islets, and glucose did not further increase islet ^{3}H-cAMP levels in the cultured islets. By contrast, 3-isobutyl-1-methylxanthine (IBMX, 1 mM) increased insulin release and ^{3}H-cAMP content in both fresh and cultured islets. Glucagon (10 μM) increased insulin release and ^{3}H-cAMP content in cultured islets better than in fresh islets; and glucagon (10 μM) plus IBMX (1 mM) increased insulin release and islet ^{3}H-cAMP content similarly in fresh and cultured islets.

It is concluded that there is rapid and, apparently, selective loss in the short-term action of glucose to increase the cAMP content in islets maintained in vitro, and this may account for the impaired insulin-releasing action of glucose observed in cultured islets. DIABETES *27*:766-73, July, 1978.

Preservation of pancreatic islets in tissue culture has attracted increasing interest during recent years. Various preparations have been described for long-term studies of pancreatic endocrine cell function in vitro. These include organ culture of fetal rat pancreas,[1-3] monolayer culture of neonatal pancreas,[4-6] and organ culture of adult isolated islets of rodents[7-14] and, recently, of man.[15,16] However, it has been shown that culturing of islets results in changes in glucose oxidation,[9] insulin content and release,[9-13] and adenylate cyclase activity.[13]

It is now well documented that glucose increases the content of adenosine 3′,5′-monophosphate (cAMP) in pancreatic islets, either isolated with collagenase from adult fed rats[17-19] or mice[20] or microdissected from obese (ob/ob) mice.[21] Impaired glucose-induced insulin release has been reported to be accompanied by reduced cAMP responses to glucose in islets of neonatal rats,[22] starved mice[20] or rats,[23] and diabetic Chinese hamsters.[24] Therefore, the aim of the present study was to determine whether the decreased stimulatory effect of glucose on insulin release in cultured islets might be associated with a deficient glucose-induced cAMP response. Accord-

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From the Institute of Clinical Biochemistry, Department of Medicine, University of Geneva, Switzerland, and the Division of Endocrinology and Metabolism, Department of Medicine, University of Miami School of Medicine, Miami, Florida.

Address reprint requests to Alexander Rabinovitch, M.D., Division of Endocrinology and Metabolism, Department of Medicine, P.O. Box 875, University of Miami School of Medicine, Miami, Florida 33152.

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ingly, insulin and cAMP responses to glucose, as well as to other agents known to increase islet levels of cAMP, were compared in freshly isolated islets and in islets that had been maintained in tissue culture medium for from several hours to six days.

MATERIALS AND METHODS

Preparation of islets. Pancreatic islets were isolated from fed male Wistar-Lewis rats weighing 200 to 250 gm. by a collagenase digestion method.[25] Each pancreas was minced into pieces about 1 to 2 mm. in size and incubated at 37° C. for five to six minutes, with vigorous shaking, in 2 ml. Hanks' balanced salt solution equilibrated to pH 7.4 with sodium bicarbonate and 95 per cent O_2:5 per cent CO_2, and it contained 3 mM glucose and 10 mg. collagenase (type I, Worthington Biochemical Corp., Freehold, N.J.). The pancreatic digest was then diluted with 10 ml. Hanks' buffer containing 3 mM glucose and washed three times, with centrifugation, in 10 ml. fresh buffer. Islets were viewed under the dissecting microscope and, by using a 10 μl. micropipette, were separated from exocrine tissues by three successive transfers into dishes containing about 20 ml. fresh buffer.

The freshly isolated islets were either incubated and studied directly (within 90 minutes of isolation from the pancreas) or first maintained in vitro as follows. One hundred fifty to 250 islets were transferred into 6-cm.-diameter plastic Petri dishes (Falcon Plastics, Oxnard, Cal.) containing 8 ml. tissue culture medium 199 (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 10 per cent heat-inactivated calf serum, antibiotics (400 I.U. per milliliter sodium penicillin G and 200 μg. per milliliter streptomycin sulfate), 14 mM sodium bicarbonate, and either 5.6 mM, 8.3 mM, or 16.7 mM glucose. The dishes were placed in a tissue incubator at 37° C. and gassed continuously with a mixture of air and 5 per cent CO_2 in order to maintain the pH of the medium between 7.35 and 7.45. The islets did not adhere to the bottom of the dish during maintenance in vitro for two hours to six days.

Perifusion experiments. For each experiment, 40 islets, freshly isolated or maintained in culture medium for 44 hours, were transferred into each of four to six perifusion chambers in parallel. The perifusion system, as applied to pieces of whole pancreas, has been previously described.[26] The basic medium was Krebs-Ringer bicarbonate (KRB) buffer continuously gassed with 95 per cent O_2:5 per cent CO_2, which was warmed to 37° C. and contained 5 mg. per milliliter dialyzed bovine serum albumin (Behringwerker A.H., Marburg, F.R.G.) and 3 mM glucose. The islets were perifused at a constant flow rate of 2.0 to 2.5 ml. per minute for an initial 30-minute equilibration period with 3 mM glucose, then stimulated for 60 minutes with 20 mM glucose, followed by a return to 3 mM glucose for 10 minutes. The effluent from each chamber was collected in one minute periods with fraction collectors, and the insulin output rate was calculated as the product of the insulin concentration measured in the effluent and the perifusion flow rate.

Labeling with ^{3}H-adenine and incubation of islets. The technique of labeling ATP in islets during a period of preincubation was adapted from Kuo and DeRenzo[27] and Humes et al.[28] The total batch of freshly isolated islets or islets that had been maintained in culture medium for two hours to six days was incubated at 37° C. for 60 minutes in 2.5 ml. KRB buffer containing 5 mg. per milliliter albumin and 3 mM glucose and 100 μCi. per milliliter ^{3}H-2-adenine (25 Ci. per mmole; New England Nuclear, Dreieichenhain, West Germany). The islets were then washed three times with 10 ml. of nonradioactive incubation buffer.

Groups of 20 islets were then incubated at 37° C. in 1.0 ml. KRB buffer containing 5 mg. per milliliter albumin and from 3 to 20 mM glucose with or without 1 mM 3-isobutyl-1-methylxanthine (IBMX, Aldrich Chemical Co., Milwaukee, Wis.) or 10 μM glucagon (lot MC 6770, Novo Research Institute, Bagsvaerd, Denmark). After three to 60 minutes of incubation, 0.1 ml. of medium was removed for insulin assay and 0.5 ml. for measuring the accumulation of ^{3}H-cAMP in the medium. Unlabeled cAMP (100 μg.) was added to the 0.5 ml. of separated medium as well as to the remaining 0.4 ml. of medium containing the islets, and the latter was boiled for five minutes. The islet content of ^{3}H-cAMP was calculated as the difference between ^{3}H-cAMP in the medium before and after boiling.

Measurements of ^{3}H-cAMP. The separation of ^{3}H-cAMP from the other radioactive substances was performed by ion exchange chromatography and barium sulfate precipitation as described by Krishna et al.[29] Briefly, the incubation media and the boiled islet extracts, each containing 100 μg. of unlabeled cAMP (as carrier), were adjusted to 1.0 ml. with water and put onto columns (4 × 0.6 cm.) of Dowex 50 × 4, 200 to 400 mesh, H^+ form (Fluka, Buchs, Switzerland) and eluted with water. cAMP was collected in the fifth and sixth 1.0-ml. fractions, and these were further treated by $BaSO_4$ precipitation of impurities,

which were removed by centrifugation. Aliquots (0.2 ml.) of the supernate were taken for determination of optical density (at 260 mm. wavelength) of the carrier cAMP added before purification, to correct for losses (50 to 60 per cent) of ^{3}H-cAMP during this procedure. The rest of the supernate (2.2 ml.) was assayed for radioactivity in 6 ml. Instagel (Packard Instrument International S.A., Zurich, Switzerland) in a Beckman liquid scintillation spectrometer (Beckman Instruments, Fullerton, Cal.). The efficiency of the above procedure for separation of ^{3}H-cAMP from radioactive substances was confirmed by phosphodiesterase treatment and measurement of the resulting ^{3}H-5′-AMP.[30]

Insulin radioimmunoassay. Insulin in islets was extracted in acid ethanol.[31] These extracts and media from incubation or perifusion of islets were diluted in glycine buffer (pH 8.6) containing 5 mg. per milliliter human serum albumin. The insulin content in these samples was measured using a charcoal separation method of radioimmunoassay.[32] The assay tracer was monocomponent porcine insulin (kindly provided by Dr. J. Schlichtkrull, Novo Research Institute) iodinated with ^{125}iodine (EIDG. Institut für Reaktorforschung, Wurenlingen, Switzerland) by the chloramine-T method[33] and purified on G50 Sephadex (Pharmacia Fine Chemicals, Uppsala, Sweden). Guinea pig antiporcine insulin antiserum (kindly supplied by Dr. P. H. Wright, University of Indiana, Indianapolis) was used as antibody, and purified rat insulin (Novo Research Institute) was used as standard in the radioimmunoassay.

Statistical methods. The significance of the difference between mean values ± S.E.M. for test and control incubations was assessed by student's *t*-test.

RESULTS

Effects of duration of culture period on islet insulin content and release and on subsequent insulin and cAMP responses to glucose. The content of insulin in islets maintained in culture medium with 8.3 mM glucose for two to 140 hours (six days) was not significantly different from that in freshly isolated islets (figure 1). There was a progressive increase in the amount of insulin released into the medium during the first 44 hours of culture; thereafter the *rate* of accumulation of insulin in the medium decreased considerably. Nevertheless, there was a progressive increase in the sum of insulin in islets plus medium, thereby suggesting a continued stimulation of insulin biosynthesis during the six days of maintaining the islets in culture medium supplemented with 8.3 mM glucose.

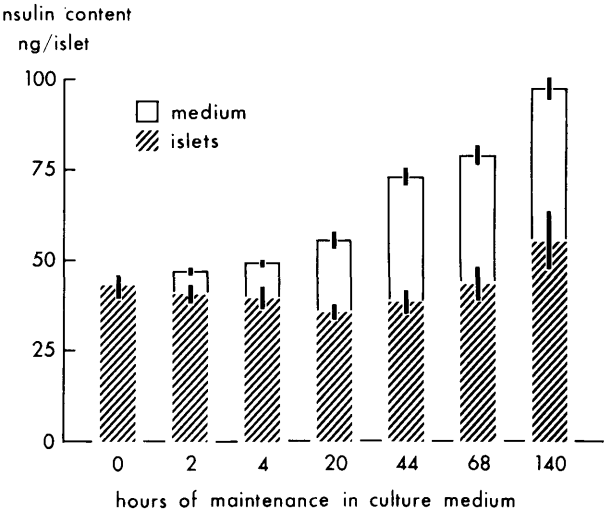

FIG. 1. Insulin content in freshly isolated islets (0 hour) and in islets and incubation medium of rat islets maintained for 2 to 140 hours (six days) in tissue culture medium containing 8.3 mM glucose. Insulin content was measured in 10 islets and in the medium in each dish at the end of the culture periods indicated. Results are mean values ± S.E.M. for five to seven dishes. The remaining islets were used for the experiments shown in figure 2.

Figure 2 compares the effects of glucose on insulin release and islet ^{3}H-cAMP content in short-term incubations (15 minutes) of freshly isolated islets and islets maintained in tissue culture medium with 8.3 mM glucose for two to 140 hours (six days). There was a progressive decrease in the ability of 20 mM glucose to stimulate insulin release in islets cultured for 2, 4, and 20 hours, and, after 20 to 140 hours of culture, glucose-induced insulin release was about one-third that in fresh islets. Similarly, 20 mM glucose stimulated a threefold increase in ^{3}H-cAMP content in fresh islets, but did not significantly increase ^{3}H-cAMP in islets cultured for only four hours or for as long as 140 hours. Basal levels of ^{3}H-cAMP (with 3 mM glucose) decreased progressively during the first 20 hours of culture, and, thereafter, ^{3}H-cAMP levels in cultured islets were about one-half the basal level observed in freshly isolated islets.

Time course of insulin and cAMP responses to glucose in fresh and cultured islets. An abrupt increase in the concentration of glucose, from 3 to 20 mM, stimulated biphasic insulin release in freshly isolated islets and also in islets that had been maintained in culture medium with 8.3 mM glucose for 44 hours (figure 3). Insulin release, integrated over the 60 minutes of perifusion with 20 mM glucose, was significantly less in the 44-hour cultured islets (116.8 ± 16.9 ng. per 40 islets, mean ± S.E.M.) than in the fresh islets (240.5 ± 17.2 ng. per 40 islets, $p < 0.01$).

Similarly, in static incubations of three to 60 minutes (figure 4), 20 mM glucose increased insulin release two- to threefold more in fresh islets than in 44-hour cultured islets. Also, 20 mM glucose significantly increased the ^{3}H-cAMP content in fresh islets incubated for from 3 to 60 minutes ($p < 0.02$), whereas ^{3}H-cAMP levels were not significantly different in cultured islets incubated with 3 or 20 mM glucose for 3 to 60 minutes.

Effects of the glucose concentration in the culture medium on islet insulin content and release and on subsequent insulin and cAMP responses to glucose. There were no significant differences in insulin content in islets maintained for 44 hours in tissue culture medium supplemented with 5.6, 8.3, or 16.7 mM glucose (table 1). Also, insulin contents in the cultured islets were not significantly less than in freshly isolated islets, although the value in islets cultured in 5.6 mM glucose (38.9 ± 3.3 ng.) approached statistical significance compared with that in fresh islets (51.4 ± 4.8 ng., $p < 0.1$). There was a progressive increase in the amount of insulin released into the culture medium over 44 hours of incubation with 5.6, 8.3, and 16.7 mM glucose, respectively.

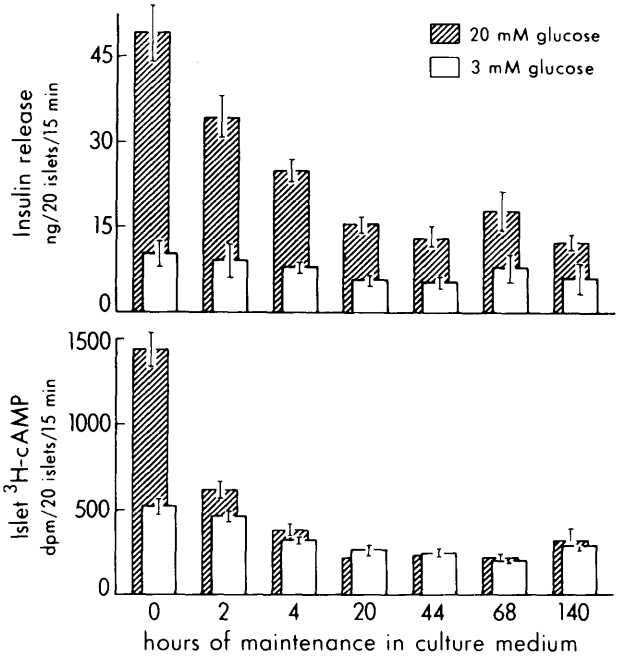

FIG. 2. Glucose-induced insulin release and ^{3}H-cAMP accumulation in freshly isolated islets (0 hour) and in islets maintained for 2 to 140 hours (six days) in culture medium containing 8.3 mM glucose. Fresh and cultured islets were preincubated with 100 μCi. per milliliter ^{3}H-2-adenine and 3 mM glucose for one hour, washed, then incubated for 15 minutes with 3 mM and 20 mM glucose. Results are mean values ± S.E.M. for 6 to 12 incubations.

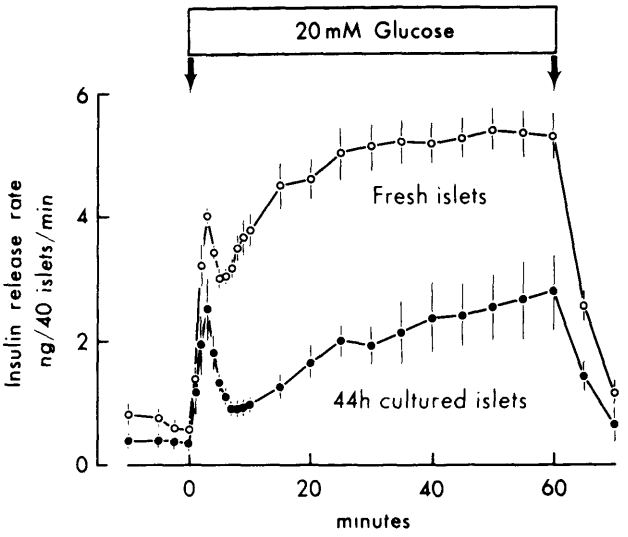

FIG. 3. Rates of insulin release from freshly isolated islets (o, mean ± S.E.M. of eight perifusions) and from islets that had been maintained for 44 hours in culture medium containing 8.3 mM glucose (•, mean ± S.E.M. of five perifusions). Forty islets were placed in each chamber and perifused with Krebs-Ringer bicarbonate buffer containing 3 mM glucose for 30 minutes (last 10 minutes shown), then stimulated with 20 mM glucose for 60 minutes followed by a return to 3 mM glucose for 10 minutes.

Figure 5 compares the dose-dependent effects of glucose on insulin release and islet ^{3}H-cAMP content in short-term incubations (30 minutes) of freshly isolated islets and islets that had been maintained for 44 hours in culture medium containing either 5.6 or 16.7 mM glucose. The sensitivity as well as the capacity of glucose-induced insulin release were less in islets cultured in either 5.6 or 16.7 mM glucose than in fresh islets. Glucose-induced insulin release in fresh islets was accompanied by a parallel dose-dependent increase in islet ^{3}H-cAMP content. By contrast, glucose did not significantly increase ^{3}H-cAMP in islets that had been cultured in medium containing either 5.6 or 16.7 mM glucose. Nevertheless, ^{3}H-cAMP levels were two- to threefold higher in islets cultured in 16.7 mM glucose rather than in 5.6 mM glucose ($p < 0.01$), and this was accompanied by increased rates of insulin release ($p < 0.05$).

Effects of glucose, glucagon, and IBMX on insulin release and cAMP content in fresh and cultured islets (table 2). Basal insulin release and islet ^{3}H-cAMP levels (with 3 mM glucose) were not significantly different in freshly isolated islets and islets cultured in 8.3 mM glucose for 44 hours. The addition of 10 μM glucagon to 3 mM glucose significantly increased insulin release and ^{3}H-cAMP content in cultured islets, whereas the increases in fresh islets were not significant. The phosphodiesterase inhibitor IBMX (1 mM) signifi-

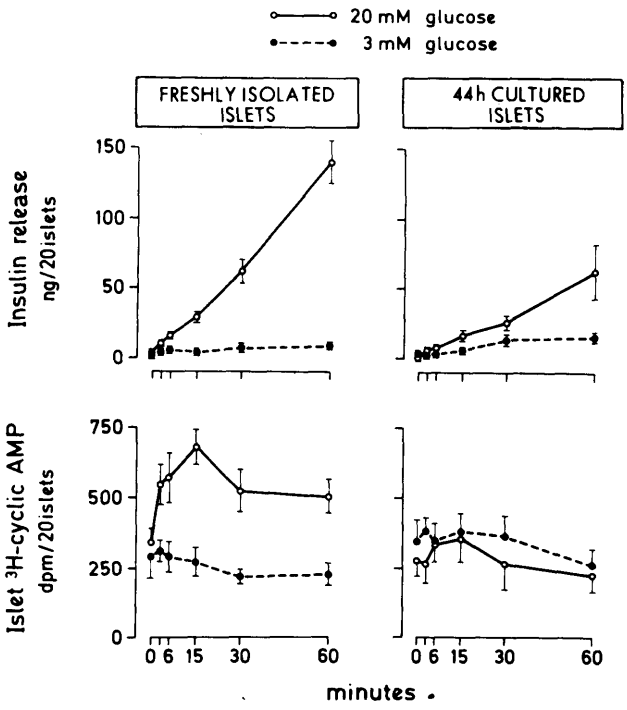

FIG. 4. Time course of glucose-induced insulin release and ^{3}H-cAMP accumulation in freshly isolated islets and in islets maintained for 44 hours in culture medium containing 8.3 mM glucose. Fresh and cultured islets were preincubated with 100 μCi. per milliliter ^{3}H-2-adenine and 3 mM glucose for one hour, washed, and then incubated with 3 mM glucose (•----•) or 20 mM glucose (o———o) for 3 to 60 minutes. Results are mean values ± S.E.M. for 8 to 10 incubations.

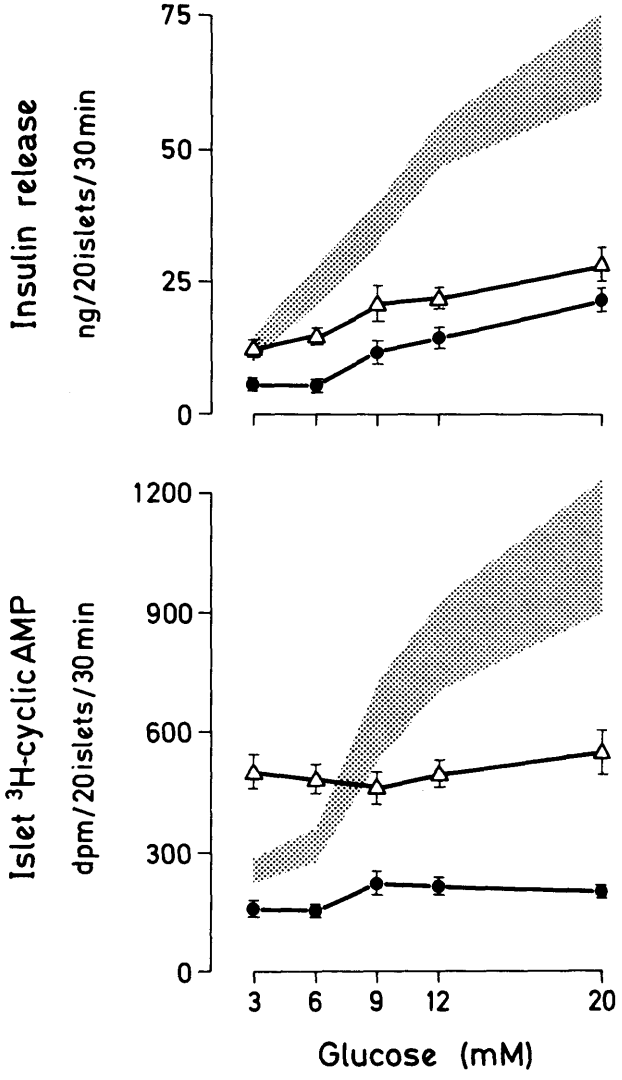

FIG. 5. Glucose dose-responses for insulin release and ^{3}H-cAMP accumulation in freshly isolated islets (shaded area) and in islets maintained for 44 hours in culture medium containing 5.6 mM glucose (•) and 16.7 mM glucose (Δ). Fresh and cultured islets were preincubated with 100 μCi. per milliliter ^{3}H-2-adenine and 3 mM glucose for one hour, washed, then incubated for 30 minutes with 3 to 20 mM glucose. Results are mean values ± S.E.M. for 6 to 12 incubations.

cantly increased insulin release and ^{3}H-cAMP content both in fresh and cultured islets, and the addition of 10 μM glucagon to 1 mM IBMX increased insulin release and ^{3}H-cAMP content similarly in fresh and cultured islets. By contrast, 20 mM glucose increased insulin release in fresh islets (+533 per cent) more than in cultured islets (+202 per cent) and increased ^{3}H-cAMP significantly in fresh islets (+284 per cent) but not significantly in cultured islets (+13 per cent). Similarly, comparison of 3 and 20 mM glucose in the presence of 1 mM IBMX revealed a greater increase in insulin release in fresh islets (+851 per cent) than in cultured islets (+241 per cent) and a significant increase in ^{3}H-cAMP in fresh islets (+175 per cent) and no increase in cultured islets.

TABLE 1

Insulin content of islets and incubation media after 44 hours of maintenance in different glucose concentrations

Glucose concentration in culture medium* (mM)	Insulin content† Islets (ng./islet)	Culture medium (ng./islet/44 hr.)
5.6	38.9 ± 3.3(7)	1.9 ± 0.5(7)
8.3	47.0 ± 3.0(8)	19.9 ± 2.0(8)
16.7	43.3 ± 3.3(7)	49.8 ± 3.7(7)
Fresh islets	51.4 ± 4.8(8)	

*150 to 250 islets were incubated for 44 hours in each dish containing 8 ml. of culture medium supplemented with glucose in the concentrations indicated.

†Insulin content was measured in 10 islets and in the medium in each dish after 44 hours of incubation. Results are mean values ± S.E.M. for the number of dishes shown in parentheses.

Effects of collagenase on insulin release and ^{3}H-cAMP content in cultured islets (table 3). In order to determine whether the glucose-induced increase in ^{3}H-cAMP

TABLE 2

Effects of glucose, glucagon, and IBMX on insulin release and ^{3}H-cAM content in fresh and cultured islets*

	Glucose concentration (mM)	Glucagon concentration (μM)	IBMX concentration (mM)	Insulin release		Islet ^{3}H-cAMP content	
				Fresh islets	Cultured islets	Fresh islets	Cultured islets
				(ng./20 islets/15 min.)		(dpm/20 islets/15 min.)	
A	3	0	0	4.9 ± 0.7	5.1 ± 0.7	264 ± 22	224 ± 25
B	3	10	0	5.3 ± 0.4	9.8 ± 1.7	304 ± 33	353 ± 54
C	3	0	1	7.0 ± 0.6	11.3 ± 1.4	1,381 ± 116	825 ± 176
D	3	10	1	13.1 ± 1.5	17.9 ± 2.9	2,774 ± 139	3,047 ± 412
E	20	0	0	31.0 ± 2.4	15.4 ± 2.0	1,014 ± 139	252 ± 41
F	20	0	1	66.6 ± 4.7	38.5 ± 5.7	3,796 ± 474	782 ± 64
Statistical treatment of data							
A versus B				N.S.	< 0.05	N.S.	< 0.05
A versus C				< 0.05	< 0.005	< 0.001	< 0.005
C versus D				< 0.05	< 0.05	< 0.001	< 0.001
A versus E				< 0.001	< 0.001	< 0.001	N.S.
C versus F				< 0.001	< 0.001	< 0.001	N.S.

*Freshly isolated islets and islets maintained for 44 hours in culture medium with 8.3 mM glucose were preincubated for one hour with 100 μCi. per milliliter ^{3}H-2-adenine and 3 mM glucose, washed, then incubated for 15 minutes with glucose, glucagon, and 3-isobutyl-1-methylxanthine (IBMX). Mean values ± S.E.M. for 9 to 11 incubations are shown and compared by student's *t*-test; N.S., not significant.

content observed in freshly isolated islets might be due to recent exposure to collagenase, 44-hour cultured islets were incubated with the collagenase preparation used to isolate islets. Glucose (20 mM) still had a small but significant effect on insulin release and no significant effect on islet ^{3}H-cAMP levels in the presence of a wide range of collagenase concentrations.

DISCUSSION

The present study confirms and extends previous observations that maintenance of pancreatic islets in tissue culture in vitro is associated with a reduced ability of glucose to stimulate insulin release in subsequent short-term incubations.[9-13] Glucose-induced insulin release decreased progressively in islets that had been cultured for 2, 4, and 20 hours and thereafter, and, for six days at least, the insulin response to glucose was about one-third that in freshly isolated islets (figure 2). Both phases of glucose-induced insulin release were reduced in islets cultured for 44 hours (figure 3). Dose-response studies with glucose indicated that the sensitivity as well as the capacity of glucose-induced insulin release were less in islets maintained for 44 hours in culture medium containing either a low (5.6 mM) or a high (16.7 mM) glucose concentration than in freshly isolated islets (figure 5).

It was therefore of interest to observe a parallel, in time, between the decreasing ability of glucose to stimulate insulin release and the loss of an effect of the sugar on the cAMP content in cultured islets (figure 2). These changes could not be accounted for by any changes in islet content of insulin (figure 1). Also, impairment of insulin and cAMP responses to glucose was not due to any adverse effects of the culture medium per se, since similar changes were seen in islets incubated for four hours in Krebs-Ringer bicarbonate buffer (data not shown).

Impairment of insulin and cAMP responses in cultured islets appeared to be selective for glucose, since glucagon and the phosphodiesterase inhibitor IBMX (alone and together) were as effective, or even more

TABLE 3

Effects of collagenase on insulin release and ^{3}H-cAMP content in cultured islets*

Collagenase concentration (μg./ml.)	Insulin release		Islet ^{3}H-cAMP content	
	3 mM glucose	20 mM glucose	3 mM glucose	20 mM glucose
	(ng./20 islets/15 min.)		(dpm/20 islets/15 min.)	
0	5.2 ± 1.1	15.5 ± 2.0	108 ± 16	124 ± 25
0.001	6.4 ± 2.8	22.7 ± 3.0	117 ± 27	174 ± 38
0.01	7.3 ± 2.1	17.5 ± 2.0	160 ± 17	143 ± 31
0.1	4.3 ± 0.8	14.5 ± 0.8	176 ± 37	118 ± 11
1	3.7 ± 0.5	16.0 ± 1.0	182 ± 49	150 ± 54
10	5.8 ± 1.9	21.7 ± 3.0	122 ± 23	126 ± 36
100	9.7 ± 2.9	18.6 ± 4.4	157 ± 25	140 ± 23

*Islets were maintained for 44 hours in culture medium with 8.3 mM glucose, incubated for one hour with 100 μCi. per milliliter ^{3}H-2-adenine and 3 mM glucose, washed, then incubated for 15 minutes with glucose ± collagenase in the concentrations shown. Mean values ± S.E.M. for four incubations are shown.

effective, in increasing insulin release and cAMP content in cultured islets than in fresh islets (table 2). These findings suggest that the responses of the effector systems for insulin release to the postulated signal derived from cAMP are unaltered by maintaining islets in vitro.

The present demonstration, that the effects of glucagon on islet cAMP content and insulin release are improved by maintaining islets in tissue culture for 44 hours after isolation, is similar to the reports that somatostatin inhibition of glucose-stimulated insulin release was markedly enhanced[34,35] and that the stimulatory effect of a hypothalamic extract on insulin release was much increased[35] by preincubating islets in culture medium for 4 to 48 hours. These findings could indicate, as suggested by others,[34-36] that islet isolation with collagenase may damage cell surface receptors and that the latter may be regenerated during a period of culture.

By contrast, the reason(s) for the *decreased* stimulatory effects of glucose on insulin release and cAMP content in cultured islets is not readily apparent. The glucose-induced increase in cAMP content in freshly isolated islets at least does not appear to be due to recent exposure to collagenase, since we could not detect any effect of collagenase (0.001 to 100 μg. per milliliter) on either basal or glucose-induced cAMP accumulation (or insulin release) in islets that had been maintained in culture medium for 44 hours (table 3). Furthermore, glucose has been reported to increase the cAMP content in islets freshly isolated by freehand microdissection from obese mice.[21]

Although glucose did not increase cAMP in short-term incubations (three to 60 minutes) of islets previously maintained in culture medium for four hours or more, the sugar had increased cAMP levels after a 44-hour culture period (figure 5). Similarly, long-term modulatory effects of glucose on adenylate cyclase activity in cultured islets have previously been reported by Howell et al.[37] and Lacy et al.[13] Therefore it appears that the acute and not the chronic effects of glucose on the adenylate cyclase-cAMP system may be deranged in cultured islets. Capito and Hedeskov[38] have recently reported an acute stimulatory effect of glucose, as well as of certain glucose metabolites, on adenylate cyclase in islet homogenates. Therefore a comparison of the effects of glucose on adenylate cyclase activity in homogenates of fresh and cultured islets may reveal whether impaired cAMP responses to glucose in cultured islets can be accounted for by defective activation of adenylate cyclase. It should be noted that the adenylate cyclase enzyme per se is intact in cultured islets, since glucagon, as well as IBMX, effectively increased islet cAMP levels.

The present observations that insulin release and islet cAMP content change in the same direction during culture, i.e., decrease in response to glucose and increase in response to glucagon, suggest that cAMP is involved in insulin secretion. However, the present study also reveals, in agreement with others,[17,21] that cAMP may be neither a necessary nor a sufficient mediator of glucose-induced insulin release. Thus, in cultured islets, glucose significantly increased insulin release without any significant increase in islet cAMP content (table 2). Also, IBMX and glucagon (in the presence of IBMX) increased cAMP in fresh and cultured islets more than did glucose, whereas the sugar was a relatively more potent stimulator of insulin release. Nevertheless, in the absence of a cAMP response to glucose, insulin release was considerably reduced (figures 2, 4, and 5). Therefore, an increase in islet cAMP may be essential for the establishment of a *normal* glucose-induced insulin release response, as was previously proposed in studies with islets of neonatal rats,[22] starved mice[20] or rats,[23] and diabetic Chinese hamsters.[24]

In conclusion, the impaired insulin-releasing action of glucose in cultured islets may be due to the loss of the ability of glucose to increase the islet concentration of cAMP. Islets maintained in tissue culture in vitro may provide a convenient preparation in which to study conditions and/or factors that might influence or be essential for the stimulation of the adenylate cyclase-cAMP system in pancreatic B-cells.

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