Myo-Inositol and the Maintenance of β-Cell Function in Cultured Rat Pancreatic Islets

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SUMMARY

We studied the effect of the *myo*-inositol content of culture media on several of the functional characteristics of cultured adult rat pancreatic islets. Variations in the ambient *myo*-inositol concentration had no effect on insulin content, its rate of synthesis, or its secretion in response to glucose when studied in freshly isolated islets. However, *myo*-inositol did inhibit the incorporation of ^{3}H-thymidine into the DNA of the fresh islet. When islets were cultured for 6 days in the absence of *myo*-inositol, their basal rate of insulin secretion was elevated and they were unresponsive to an elevated glucose concentration. As the *myo*-inositol content of the culture medium was increased from 10 to 40 μg/ml, a progressive decrease in basal insulin secretion as well as an increase in glucose-stimulated insulin secretion was observed. Similarly, the total insulin content of cultured islets increased progressively as the *myo*-inositol content of the culture medium was raised. The rates of incorporation of ^{3}H-leucine into islet protein, proinsulin, insulin, and C-peptide were found to be twice as high in the presence of 40 μg/ml *myo*-inositol when compared with the rates observed in the absence of this material. The incorporation of ^{3}H-thymidine into the DNA of cultured islets was maximal when the medium contained 20 μg/ml *myo*-inositol, and was decreased when concentrations above or below this value were employed. These results demonstrate that the ambient *myo*-inositol concentration to which cultured islets are exposed in vitro exerts a strong influence on their functional characteristics. Thus, the *myo*-inositol content of the culture media employed should be taken into consideration in the interpretation of any studies involving cultured pancreatic islets. DIABETES *30*:621–625, August 1981.

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Tissue culture of isolated pancreatic islets has proved to be a useful tool for the elucidation of those factors that are required for the long-term maintenance of the ability of the β-cells to synthesize and secrete insulin. The influence of glucose on β-cell secretion,[1–7] metabolism,[1,4] insulin biosynthesis,[1,2,6,8] replication,[8–10] electrical activity,[11] and generation of cyclic AMP[5] has been amply documented. In general, these studies indicate that a moderately high level of glucose is required to maintain competent islets in culture. In addition, serum also has been found to favor the maintenance of β-cell function.[6] While some investigators have found that serum-free medium supplemented with high glucose will sustain islet function, high glucose by itself has not been found to be sufficient to maintain optimal islet function.[3,12] Thus, it is generally accepted that serum provides hormones and/or nutrient factors that are necessary for the growth and survival of cultured cells.[13] It is conceivable that the partial preservation of islet function in serum-free medium containing high glucose is due to endogenous insulin secretion, since this hormone is required for the maintenance of optimal growth and functional characteristics of several endocrine cell lines.[13]

Andersson[6] has compared the ability of several different culture media to support insulin biosynthesis and the secretory capacity of cultured mouse islets in the presence or absence of serum. He found that the functional integrity of islets was better maintained when they were cultured in RPMI 1640 than when they were cultured in other media. He further observed that the superiority of the medium was not due to its high glucose or nicotinamide content. We noted that the most striking difference between the nutrient content of RPMI 1640 and that of the other media that he tested was in their relative concentrations of *myo*-inositol. In view of the fact that 10% serum also contributes a substantial amount of *myo*-inositol to the culture media, we studied the influence of this nutrient on several functional parameters of adult rat islets cultured in the absence of serum and in the presence of a high glucose concentration.

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MATERIALS AND METHODS

Animals and tissue culture. Pancreatic islets, isolated by collagenase treatment and a Ficoll gradient,[14] were obtained from male Sprague-Dawley rats (200–300 g). Batches of islets were either studied immediately or suspended in 1 ml of tissue culture medium in 35-mm plastic Petri dishes. The standard medium used was CMRL 1066, which had been prepared without either *myo*-inositol or glucose. The basic medium was supplemented with 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin sulfate. The glucose content of the medium was adjusted to 11.1 mM for the 6-day culture period and to other concentrations as described in the text as required for the individual experiments. When *myo*-inositol was present, it was added over the range of 10–40 μg/ml. The cultures were maintained at 37°C in a humidified atmosphere of air and 5% CO_2. The islets were cultured for 6 days before use in an experiment, and the medium was replaced with fresh medium of identical composition on day 5.

Measurement of *myo*-inositol. The *myo*-inositol content of the various culture media and of fetal calf serum was determined by gas-liquid chromatography of the trimethylsilyl ether derivative of *myo*-inositol, as previously described.[16]

Insulin secretion and content. Groups of 10–15 fresh or cultured islets were placed in 10 × 75-mm tubes containing 0.5 ml of Krebs-Ringer bicarbonate buffer[15] supplemented with 3.0 mg/ml bovine serum albumin and 5.6 or 16.7 mM glucose, and incubated for 60 min at 37°C with a gas phase of 95% O_2 and 5% CO_2. At the end of the incubation period, the medium was removed and stored at −20°C before insulin assay using a single-antibody radioimmunoassay technique with porcine insulin as a standard.[17]

Islet insulin content was measured in groups of 10–15 islets after sonication in 0.5 ml of acid-ethanol (ethanol, HCl, water; 96:2.4:18, vol/vol/vol) and extraction overnight at 4°C. The extracts were stored at −20°C before insulin assay.

Insulin, proinsulin, C-peptide, and protein biosynthesis. Groups of 10 freshly isolated or cultured islets were transferred to 10 × 12-mm plastic center wells for determination of protein, proinsulin, insulin, and C-peptide biosynthesis. Each tube contained 0.25 ml of Gay and Gay bicarbonate-buffered medium containing 12 naturally occurring amino acids but with the omission of L-leucine.[18] The incubation medium was supplemented with 2.0 mg/ml bovine serum albumin, 5.6 or 27.8 mM glucose, 50 μCi/ml L-[4,5-^{3}H]-leucine, and 0–40 μg/ml *myo*-inositol. In the case of cultured islets, the *myo*-inositol concentration to which they were exposed during the experimental period was the same as that employed during the 6 days of culture. The islets were incubated for 3 h at 37°C in an atmosphere of 95% air and 5% CO_2. At the end of the incubation period, the islets were washed three times with 0.15-ml portions of a phosphate-buffered saline solution containing 10 mM unlabeled L-leucine and then were disrupted by sonication. The recovery of labeled protein, proinsulin, insulin, and C-peptide was determined by separation of these peptides on a calibrated Sephadex G-50 column, and the individual fractions were counted by liquid scintillation spectrometry.

Estimation of DNA synthesis. Groups of 10 fresh or cultured islets were transferred to plastic tubes containing 0.5 ml of standard culture medium supplemented with *myo*-inositol and glucose as required. After the addition of 10 μCi/ml of [methyl-^{3}H]-thymidine to each group of islets, the tubes were incubated for 3 h at 37°C in a humidified atmosphere of air containing 5% CO_2. After incubation the islets were washed 3 times over a period of 5 h with 0.5-ml portions of culture medium containing 5 mg of cold thymidine. The islets were subsequently washed with 0.5 ml Hanks' saline solution 3 times before ultrasonic disruption in 0.1 ml of distilled water. The radioactivity was determined by liquid scintillation spectrometry.

Chemicals. RPMI 1640 and CMRL 1066 (without glucose or *myo*-inositol), glutamine, penicillin-streptomycin, fetal calf serum, and thymidine were obtained from Gibco (Grand Island, New York). Collagenase and *myo*-inositol were obtained from Sigma Chemical Co. (St. Louis, Missouri). L-[4,5-^{3}H]-leucine and [methyl-^{3}H]-thymidine were obtained from Amersham (Arlington Heights, Illinois). Sephadex G-50 was obtained from Pharmacia (Piscataway, New Jersey).

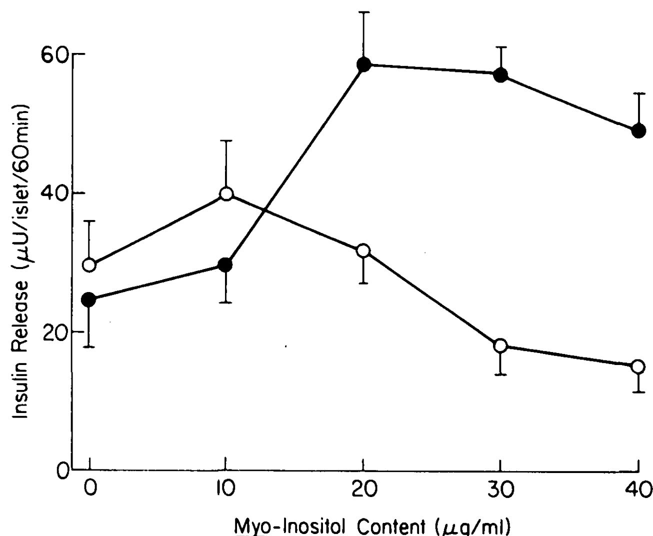
Statistical methods. All results are given as mean ± SEM. Statistical differences were determined by the two-tailed Student's *t* test.

RESULTS

***Myo*-inositol content of incubation media.** The *myo*-inositol contents of various batches of CMRL 1066, 10% fetal calf serum, CMRL 1066 plus 10% fetal calf serum, RPMI 1640, and RPMI 1640 plus 10% fetal calf serum were found to be 0.2 ± 0.1, 14.9 ± 0.6, 15.5 ± 0.5, 35.1 ± 1.7, and 46.0 ± 2.4 μg/ml, respectively (in each instance N = 6). Consequently, the range of *myo*-inositol concentrations used in all subsequent experiments was from 0 to 40 μg/ml. No *myo*-inositol was detected in the *myo*-inositol-free CMRL 1066 prepared for us by Gibco.

Insulin secretion and content. Variations in the ambient *myo*-inositol content (over the range of 0–40 μg/ml) had no effect on the basal or stimulated secretion of insulin from

FIGURE 1. Influence of *myo*-inositol content of the culture medium on the secretory response of islets that had been cultured in 11.1 mM glucose for 6 days. At the end of the culture period, the medium was replaced with Krebs-Ringer bicarbonate buffer containing 3.0 mg/ml BSA and 5.6 (-○-) or 16.7 (-●-) mM glucose. Groups of 10–15 islets were incubated for 1 h, after which the medium was removed for determination of insulin content. Each point represents the mean of 9 observations and the vertical bars represent the SEM.



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freshly isolated rat islets or on their total insulin content (data not shown).

The ability of 5.6 versus 16.7 mM glucose to stimulate insulin release from islets previously cultured for 6 days in medium containing 11.1 mM glucose and with varying concentrations of *myo*-inositol is shown in Figure 1. There was no significant difference in the secretory response elicited by 16.7 mM glucose compared with that obtained with 5.6 mM glucose when the islets were exposed to either 0 or 10 μg/ml *myo*-inositol during the culture period. When islets were cultured in 20 μg/ml *myo*-inositol, high glucose elicited a 1.8-fold increase in the secretory response compared with the basal secretory rate. There was more than a threefold increment in the glucose-induced secretory response observed with islets that had been cultured in either 30 or 40 μg/ml *myo*-inositol. The basal insulin release appeared to decline progressively as the *myo*-inositol content of the culture medium was increased from 10 to 40 μg/ml.

When islets were cultured in medium containing 11.1 mM glucose with 0 or 10 μg/ml *myo*-inositol, there was no difference in their total insulin content (Table 1). However, as the *myo*-inositol content of the culture medium was increased to 20 μg/ml or greater, there appeared to be a progressive increase in the recovered islet insulin content. The insulin content of islets cultured with 11.1 mM glucose and 40 μg/ml *myo*-inositol was approximately 70% of the insulin content of freshly isolated islets (data not shown).

Insulin biosynthesis. Variation in the ambient concentration of *myo*-inositol was found to have no influence on the rates of void-volume protein, proinsulin, insulin, or C-peptide synthesis in freshly isolated rat islets (data not shown).

The short-term rates of protein, proinsulin, insulin, and C-peptide biosynthesis observed with islets that had been cultured in media containing 11.1 mM glucose and 0, 20, and 40 μg/ml *myo*-inositol are shown in Figure 2. There was no statistically significant difference in the rate of synthesis of each moiety when islets were exposed to either 5.6 or 16.7 mM glucose in acute experiments after the 6-day culture period (data not shown). The rate of incorporation of ^{3}H-leucine into the various measured peptides was observed to be similar in islets that had been cultured either in the absence of *myo*-inositol or in the presence of this compound at a concentration of 20 μg/ml. Compared with that obtained without *myo*-inositol, the incorporation of ^{3}H-leucine into the proinsulin-insulin pool was more than doubled when islets had been cultured in 40 μg/ml *myo*-inositol. There was a similar increment in total islet protein synthesis due to the presence of 40 μg/ml *myo*-inositol during the culture period.

TABLE 1
Influence of the concentration of *myo*-inositol in the culture medium on islet insulin content

Myo-inositol content (μg/ml)	Islet insulin content (mU/islet)	P value
0	0.49 ± 0.19	—
10	0.56 ± 0.06	NS
20	1.05 ± 0.08	< 0.05
30	1.28 ± 0.05	< 0.005
40	1.60 ± 0.12	< 0.001

Islets were cultured for 6 days in medium containing 11.1 mM glucose and the *myo*-inositol concentrations indicated. After culture the islets were washed, homogenized in acid-ethanol, and extracted overnight at 4°C. Eight batches of islets were studied under each of the experimental conditions. The P values indicate the level of significance of a given value versus 0 μg/ml *myo*-inositol.

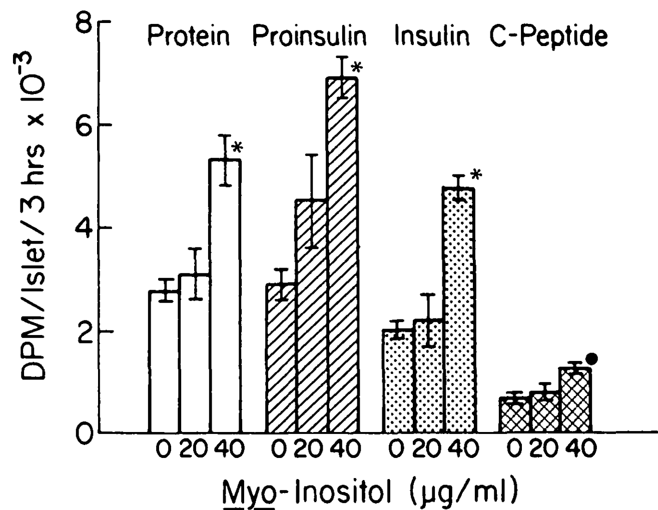

FIGURE 2. Effect of the *myo*-inositol content of the culture medium on the incorporation of ^{3}H-leucine into void-volume protein, proinsulin, insulin, and C-peptide. After 6 days of culture in medium containing 11.1 mM glucose and the indicated concentrations of *myo*-inositol, the islets were transferred to tubes containing culture media supplemented with ^{3}H-leucine, 5.6 mM glucose, and the indicated concentrations of *myo*-inositol. After 3 h, the islets were washed, sonicated, and the sonicate was applied to a 100-cm column of Sephadex G-50 that had been previously calibrated with rat proinsulin, rat insulin, and with porcine glucagon. The radioactivity of the various fractions was determined by liquid scintillation spectrometry and the total radioactivity contained within each peak was estimated by triangulation. Each column represents the mean of 10–12 determinations and the vertical bars represent the SEM. *P < 0.001; • P < 0.005.

Estimation of islet DNA synthesis. The rate of incorporation of ^{3}H-thymidine into the DNA of fresh islets was found to be 162.2 ± 49.0 dpm/islet/3 h (N = 10). The rate of incorporation was decreased by more than 66% when *myo*-inositol in a concentration of 10 μg/ml or greater was present (data not shown), suggesting that *myo*-inositol has a toxic effect on DNA synthesis in the fresh islet.

As was observed in the studies of incorporation of ^{3}H-leucine into cultured islet proteins, we were unable to observe any effect of acute alterations of the ambient glucose concentration on the rate of incorporation of ^{3}H-thymidine into DNA in freshly isolated islets (data not shown). In contrast with what was observed with fresh islets, there was no significant difference in the rates of ^{3}H-thymidine incorporation when islets were cultured in 0, 10, 30, or 40 μg/ml *myo*-inositol (Figure 3). The highest rate of ^{3}H-thymidine incorporation into islet DNA was obtained when islets were cultured for 6 days in medium containing 20 μg/ml *myo*-inositol. These results demonstrate a clear difference between the optimum *myo*-inositol concentration required for the maximum rates of proinsulin-insulin and DNA synthesis in cultured islets. Whereas the maximum rate of DNA synthesis was observed at a *myo*-inositol concentration of 20 μg/ml and was decreased by further incremental increases, the rate of the islet proinsulin-insulin synthesis, the insulin content, and the ability of the islets to secrete insulin in response to glucose were all observed to increase progres-

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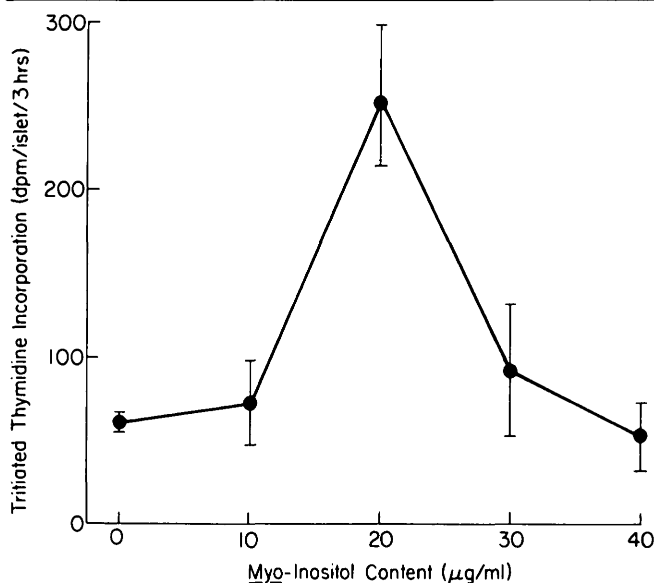

FIGURE 3. Influence of *myo*-inositol on DNA synthesis in islets previously cultured for 6 days in CMRL 1066 medium containing 11.1 mM glucose and the indicated concentration of *myo*-inositol. Islets were transferred to tubes containing culture medium supplemented with *myo*-inositol and ^{3}H-thymidine. After 3 h the islets were extensively washed with culture medium followed by Hanks' saline solution before sonication. The radioactivity was determined by liquid scintillation spectrometry. Each point represents the mean value of 6 replicate determinations and the vertical bars represent the SEM.

sively as the *myo*-inositol content of the culture medium was increased.

DISCUSSION

In short-term studies, the maintenance of isolated pancreatic islets in tissue culture has been found to require a high concentration of glucose to preserve glucose-induced insulin release and other associated events.[1-12] It has been suggested that the effect of glucose results from the protection of a putative β-cell glucoreceptor mechanism that is involved in the initiation of insulin release.[7] Although the presence of 10% serum is necessary to sustain optimal β-cell function, the presence of a high glucose concentration in serum-free medium is sufficient to maintain the secretory potential of β-cells.[7,12] Interestingly, it has been found that, among several tissue culture media tested, RPMI 1640 is by far the most effective in maintaining not only the insulin synthetic capacity of cultured islets, but also their secretory capacity in acute experiments.[6] The superiority of RPMI 1640 could not be reproduced when TCM 199 was supplemented with glucose and nicotinamide at the concentrations that are contained in RPMI 1640. Another striking difference in the composition of RPMI 1640 in relation to most other culture media is its high content of *myo*-inositol (35 μg/ml) compared with less than 1 μg/ml of this material in TCM 199 or CMRL 1066 media. Thus, it occurred to us that *myo*-inositol may have been the nutrient factor contained in serum or RPMI 1640 that contributed to the maintenance of optimal β-cell function in the cultured islet.

Our results indicate that *myo*-inositol is indeed an important constituent of the culture media in which pancreatic islets are maintained. In the absence of serum and in the presence of 11.1 mM glucose, at least 20 μg/ml *myo*-inositol was required to sustain the secretory capacity of glucose-stimulated islets as well as DNA synthesis. However, the optimal rate of insulin biosynthesis was obtained at 40 μg/ml *myo*-inositol, whereas 20 μg/ml supported maximal DNA synthesis. Other investigators have also found that the sensitivities of islet DNA and insulin biosynthesis in response to variations in the nutrient content of the culture medium are different.[6,8]

Despite the presence of 10% calf serum in culture medium TCM 199 or CMRL 1066 (both of which contain 5.6 mM glucose and a negligible level of *myo*-inositol), the secretory capacity and insulin content does not achieve that of islets cultured with RPMI 1640 containing 11 mM glucose.[6] Since the *myo*-inositol content of such media would be less than 20 μg/ml, it is possible that this concentration of *myo*-inositol was below the threshold concentration of this substance required to support the synthesis or the secretion of insulin by the cultured islet. Other groups have found that much higher levels of glucose (i.e., 17–20 mM) will greatly enhance the maintenance of β-cell function in serum-free medium despite the presence of only a low concentration of *myo*-inositol.[6,7] Because it is well known that high glucose is capable of sustaining islet function in serum-free medium, the glucose content of all culture media used in the present experiments was maintained at a high concentration to determine the extent to which *myo*-inositol, by itself, could further enhance the function of the β-cells in culture. The results of these studies reveal that the *myo*-inositol content of the culture medium has profound effects on the synthesis and secretion of insulin that are independent of the effect of glucose on these phenomena. Furthermore, they suggest that it is the high *myo*-inositol content of RPMI 1640 that accounts for the superiority of this medium for the long-term storage of isolated islets when compared with other commercially available media.

Although it has long been recognized that exogenous *myo*-inositol is an essential growth factor for most mammalian cells in tissue culture,[19] the molecular mechanisms responsible for this role are unknown. Since rat islets lack the capacity to oxidize this compound (Clements and Rhoten, unpublished data), it is unlikely that *myo*-inositol serves as an important fuel for this tissue in culture. It is more likely that the provision of exogenous *myo*-inositol is required for the maintenance of normal islet concentrations of phosphatidylinositol. This membrane-associated phospholipid is synthesized within the rat pancreatic islet, and the turnover of islet phosphatidylinositol has recently been speculated to play a role in glucose-stimulated insulin secretion.[20,21] Thus, it is possible to speculate that the provision of adequate substrate for islet phosphatidylinositol synthesis may be required for the maintenance of both the glucose sensitivity as well as the insulin synthetic capacity of the cultured rat islet.

Obviously, the mechanisms by which phosphatidylinositol metabolism might influence either the synthesis or the secretion of insulin by the cultured islet remain unclear. With regard to the effect of *myo*-inositol on DNA synthesis, it has recently been proposed that the metabolism of *myo*-inositol-containing phospholipids may play an important role in the regulation of cell growth and division in rat intestinal mucosa, human lymphocytes, and cultured rat fibroblasts.[22,23] If a similar situation exists in cultured rat islet cells, it may explain the observed effect of *myo*-inositol on DNA synthesis in the present studies. Regardless of the mechanisms in-

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volved, it is clear that the ambient *myo*-inositol concentrations to which cultured rat islets are exposed in vitro can profoundly influence their functional characteristics, and that the ambient concentrations of this compound can exert a major influence on the experimental results obtained in any study involving islet culture. The present results may explain, in part, the discrepant results obtained in previous studies of the functional properties of cultured islets, and suggest that the *myo*-inositol content of the culture medium must be taken into consideration in both the design and in the interpretation of such studies.

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