Effects of D-glucose, L-leucine, and 2-ketoisocaproate on Insulin mRNA Levels in Mouse Pancreatic Islets

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SUMMARY

To elucidate a possible mechanism for regulation of insulin mRNA levels in the pancreatic B-cell, isolated mouse pancreatic islets were cultured in the presence of either glucose, leucine, or 2-ketoisocaproate, and insulin mRNA levels were compared with insulin biosynthesis, insulin release, and islet O_2 uptake. It was observed that leucine or 2-ketoisocaproate was as effective as 20 mM glucose in supporting high insulin mRNA levels, high basal rates of insulin release or insulin synthesis, and rapid O_2 uptake. Furthermore, islets cultured with either leucine or 2-ketoisocaproate could be stimulated to increase their insulin biosynthesis by a high glucose concentration. In addition the insulin release and respiration of such islets could be increased by exposure to 2-ketoisocaproate + glutamine. It is concluded that the maintenance of high concentrations of insulin mRNA levels and high rates of insulin biosynthesis and release are all processes correlated with metabolic fluxes in islets rather than the presence of the glucose molecule per se. DIABETES 1986; 35:228–31.

Glucose stimulates the rate of insulin biosynthesis in the islets of Langerhans.[1] Although short-term experiments indicate that this stimulation occurs at a posttranscriptional level,[2,3] long-term glucose deprivation causes a significant reduction of islet insulin mRNA levels.[4,5] Thus far, it has not been determined unequivocally whether this reduction is due to decreased transcription of insulin mRNA or to altered stability of insulin mRNA, or both.

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Received for publication 24 August 1984 and in revised form 30 December 1984.

Both leucine, and its deamination product 2-ketoisocaproate, stimulate insulin biosynthesis.[6,7] Since both compounds increase catabolic fluxes in islets,[8] their mode of action is compatible with the view that stimulation of insulin biosynthesis is the result of increased islet metabolism.[1,9] Furthermore, leucine supports high rates of insulin production during culture of isolated islets.[10]

The present investigation was undertaken to determine whether the maintenance of high insulin mRNA levels during culture of islets specifically requires high glucose concentrations, or whether elevated insulin mRNA concentrations may be related to overall islet metabolic fluxes. To test this possibility, the islet insulin mRNA content was determined after culture of islets in the presence of either 2-ketoisocaproate or leucine, two nongluconeogenic compounds.[11] Furthermore, islet oxidative metabolism (measured as islet O_2 uptake), insulin biosynthesis, and insulin release were determined under similar conditions.

MATERIALS AND METHODS

Materials. L-leucine, 2-ketoisocaproate, and Hepes (2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid) were from Sigma Chemical Co., St Louis, Missouri. D-glucose was from Mallinckrodt, St Louis, Missouri. (α-^{32}P)dCTP (3000 Ci/mmol) and (2,4,6-^{3}H)-phenylalanine (50 Ci/mmol) were from Amersham, Bucks, United Kingdom. (I^{125}) insulin was obtained from New England Nuclear, Boston, Massachusetts. Tissue culture medium RPMI 1640 was obtained from Flow Laboratories, United Kingdom. Calf serum was purchased from Statens Bakteriologiska Laboratorium, Stockholm, Sweden.

Preparation and culture of isolated islets. The islets of Langerhans were isolated from male NMRI mice (Anticimex, Stockholm) by a collagenase digestion technique.[12] The islets were cultured free floating[13] in RPMI 1640 + 10% calf serum containing 3.3 mM glucose, 20 mM glucose, 3.3 mM glucose + 10 mM 2-ketoisocaproate or 3.3 mM glucose + 10 mM leucine for 7 days with one change of culture medium.

Insulin mRNA content. After 7 days of culture, equal-size groups of 35–100 islets each were collected from all the

TABLE 1
Effects of glucose or 2-ketoisocaproate + glutamine on insulin release from islets cultured under various conditions

Culture conditions	Insulin release (ng/5 islets)		
	1.5 mM glucose	16.7 mM glucose	10 mM 2-ketoisocaproate + 10 mM glutamine
3.3 mM glucose	5.4 ± 1.5 (7)*	5.1 ± 1.1 (7)	—
20 mM glucose	17.0 ± 2.3 (7)	31.9 ± 1.5† (6)	—
3.3 mM glucose + 10 mM 2-ketoisocaproate	14.5 ± 10.7 (7)	19.9 ± 1.2 (7)	32.7 ± 2.6† (7)
3.3 mM glucose + 10 mM leucine	12.7 ± 2.9 (6)	16.2 ± 1.1 (6)	32.0 ± 3.0† (6)

Islets cultured under conditions given in the left column were incubated for 1 h in the presence of either 1.5 or 16.7 mM glucose, or 10 mM 2-ketoisocaproate + 10 mM glutamine. Samples were taken for insulin assay.
*Values are means ± SEM for the number of separate islet incubations given in parentheses.
†$P < 0.001$ when tested against insulin release at 1.5 mM glucose with a two-tailed *t*-test.

different culture conditions. The islets were sonicated and the RNA was extracted in phenolchloroform, bound to diazobenzyloxymethyl (DBM)-paper squares and hybridized with a nick-translated rat insulin I cDNA probe (pRI-7) as previously described in detail.[4] The insulin mRNA levels are expressed as cpm after subtraction of background hybridization. Northern blot analyses[14] of islet RNA prepared by this method revealed no degradation of insulin mRNA.

Insulin release. Groups of approximately equal-size islets from the different culture conditions were incubated in 250 μl of a Krebs-Ringer bicarbonate buffer[15] supplemented with 2 mg/ml bovine serum albumin, 10 mM Hepes (pH 7.4), and the various additions given in Table 1 at 37°C in a 95% O_2/5% CO_2 atmosphere for 1 h. Samples were collected and the insulin concentrations were determined as described before.[16]

Insulin biosynthesis. Ten to twenty cultured islets were incubated for 2 h in 95% O_2/5% CO_2 at 37°C in Krebs-Ringer Hepes buffer containing the various additions indicated in Table 2 and 50 μCi/ml of (2,4,6-^{3}H)phenylalanine. The islets were then washed in nonradioactive buffer, transferred to distilled water (10 islets/100 μl), sonicated, and frozen until the incorporation into either insulin or total TCA precipitable protein was determined.[17] Determinations of islet DNA were made with a fluorometric method according to Kissane and Robins.[18] The average islet DNA content was in the range of 21–30 ng/islet and there were no significant differences between the groups.

Islet oxygen uptake. After culture, groups of 10 islets were transferred to Cartesian divers and incubated for approximately 1 h at 37°C in ambient air in Krebs-Ringer Hepes buffer (pH 7.4) with equimolar amounts of NaCl substituted for $NaHCO_3$. A side drop containing additions as given below was then mixed and islet respiration was followed for an additional hour. The islets were then recovered from the divers and their DNA contents determined as above. A detailed account of the technique has been given before.[19]

Changes in respiratory rates due to the addition of substrates are expressed as a percentage of the initial respiration in the absence of substrate. Rates of respiration remained linear with time for more than 2 h.

Statistical analyses. Unless otherwise stated, probabilities (P) of chance differences between groups are calculated according to Student's *t*-test.

RESULTS

Insulin mRNA content. Islets cultured for 7 days in the presence of 3.3 mM glucose displayed markedly lower insulin mRNA content than islets cultured in the presence of 20 mM glucose (Table 3). After culture with either 10 mM 2-ketoiso-

TABLE 2
Effects of glucose on (pro)insulin biosynthesis in islets cultured under various conditions

Culture conditions	PI-I (dpm/islet)		TCA (dpm/islet)		PI-I × 100/TCA	
	0.0 mM glucose	16.7 mM glucose	0.0 mM glucose	16.7 mM glucose	0.0 mM glucose	16.7 mM glucose
3.3 mM glucose	34 ± 21	97 ± 37	827 ± 138	1704 ± 386*	3.8 ± 1.6	6.0 ± 1.5
20 mM glucose	213 ± 30	366 ± 54†	1654 ± 151	2102 ± 582	13.2 ± 1.6	17.3 ± 1.4
3.3 mM glucose + 10 mM 2-ketoisocaproate	176 ± 50	362 ± 26†	1853 ± 566	2222 ± 320	9.6 ± 1.0	19.4 ± 2.0*
3.3 mM glucose + 10 mM leucine	192 ± 43	346 ± 70‡	1437 ± 226	1704 ± 195	13.3 ± 1.4	19.5 ± 1.9*

Islets cultured for 7 days under conditions given in the left column were incubated in a Krebs-Ringer bicarbonate buffer supplemented with 10 mM Hepes (pH 7.4), 50 μCi/ml (2,4,6-^{3}H) phenylalanine in the absence or presence of 16.7 mM glucose for 2 h at 37°C in a 95% O_2/5% CO_2 atmosphere. The incorporation of radioactivity into (pro)insulin (PI-I) or total protein (TCA) is shown, as well as the percentage of PI-I in total TCA precipitable protein. Means ± SEM for 6–7 experiments are given.
*$P < 0.05$ and †$P < 0.01$ when tested against the value for no glucose addition with a two-tailed *t*-test.
‡$P < 0.05$ when tested against the value for no glucose addition with a paired *t*-test.

TABLE 3
Insulin mRNA content of pancreatic islets exposed in tissue culture to glucose, 2-ketoisocaproate or leucine

Culture conditions	3.3 mM glucose	20 mM glucose	3.3 mM glucose + 10 mM 2-ketoisocaproate	3.3 mM glucose + 10 mM leucine
Insulin mRNA (cpm/50 islets)	344 ± 122	1805 ± 264†	1943 ± 362†	1492 ± 326*

Freshly isolated mouse islets (100–200) were cultured in RPMI and exposed to the various additions for 7 days after which 50 islets were sonicated in a phenolchloroform solution for extraction of RNA. The RNA was bound to DBM paper and then hybridized with a ^{32}P-labeled insulin cDNA probe. The values are given as means ± SEM for 11 separate experiments, each comprising groups of islets cultured at the four different conditions.
*$P < 0.01$ and †$P < 0.001$ versus the insulin mRNA content of islets cultured at 3.3 mM glucose using a two-tailed *t*-test.
The average background hybridization was 356 cpm. This value has been subtracted from the figures in the table.

caproate or leucine in the presence of 3.3 mM glucose, the insulin mRNA content was maintained at the same levels as in the presence of 20 mM glucose only.

Insulin release. Glucose failed to stimulate insulin release from islets cultured at 3.3 mM glucose for 7 days alone or with 2-ketoisocaproate or leucine (Table 1). Conversely, high-glucose cultured islets displayed higher rates of insulin release in the presence of 16.7 mM glucose than in its absence. In the islets cultured in the presence of leucine or 2-ketoisocaproate, insulin release was increased when 2-ketoisocaproate + glutamine were added in the short-term incubations. The insulin release at 1.5 mM glucose from islets cultured at 3.3 mM glucose was markedly lower than that from islets cultured under any other condition ($P < 0.05$).

Insulin biosynthesis. Rates of insulin biosynthesis were stimulated by 16.7 mM glucose in islets cultured for 7 days in the presence of either 20 mM glucose, 3.3 mM glucose + 10 mM 2-ketoisocaproate, or 3.3 mM glucose + 10 mM leucine (Table 2). Furthermore, the insulin biosynthesis as a percentage of total protein synthesis appeared increased by glucose in islets cultured in the above media although the values attained statistical significance only in the two latter conditions. The islets cultured in 3.3 mM glucose did not respond to glucose with increased rates of insulin biosynthesis ($P > 0.05$), although rates of total protein synthesis were significantly increased ($P < 0.05$). Moreover, the basal rates of insulin biosynthesis were lower in islets cultured in 3.3 mM glucose than in those cultured with high glucose, 2-ketoisocaproate, or leucine ($P < 0.05$). In islets cultured in the two latter conditions, the effects of 2-ketoisocaproate + glutamine on insulin biosynthesis were smaller than those of 16.7 mM glucose (data not shown).

Islet respiration. Islets cultured for 7 days in the presence of 3.3 mM glucose alone displayed a lower ($P < 0.01$) basal respiratory rate than those cultured at a similar glucose concentration with either 2-ketoisocaproate or leucine (Table 4). The exposure of islets to high glucose or 2-ketoisocaproate + glutamine after culture at either a low or a high glucose concentration caused an increase in the islet respiratory rate.

DISCUSSION

The present study demonstrates that leucine and 2-ketoisocaproate, which are nongluconeogenic in islets due to lack of islet fructose 1,6-diphosphatase,[11] were as effective in maintaining high insulin mRNA levels as was 20 mM glucose. This effect may be mediated via a common metabolic signal that could be a triosephosphate, a citric acid intermediate, or a cofactor such as ATP, GTP, cAMP, or NAD(P)H.[20] The present close agreement between the rate of islet respiration and insulin mRNA levels, indeed, suggests that the coupling between the insulin mRNA content and exogenous substrates is related to the generation of NADH. The data also confirm that insulin biosynthesis is related to the abundance of insulin mRNA present in the islets.

On the basis of the present data it could not be ascertained whether the maintenance of a high insulin mRNA content in

TABLE 4
Effects of substrates on islet respiration

Culture conditions	Basal respiration	Islet respiration (nl O_2/μg DNA) 16.7 mM glucose	10 mM 2-ketoisocaproate + 10 mM glutamine	Increment (percent of basal)
3.3 mM glucose	199 ± 26	310 ± 30*	—	57 ± 6%‡
20 mM glucose	382 ± 34	554 ± 53*	—	48 ± 15%†
3.3 mM glucose + 10 mM 2-ketoisocaproate	432 ± 51	—	860 ± 140*	98 ± 16%‡
3.3 mM glucose + 10 mM leucine	539 ± 89	—	888 ± 128†	98 ± 34%

Islets cultured under conditions given in the left column were placed in Cartesian divers and allowed to respire for approximately 60 min in Krebs-Ringer Hepes buffer only (endogenous respiration) and subsequently in the same buffer containing either glucose or 2-ketoisocaproate + glutamine. The increment in respiration was estimated as a percentage of the endogenous respiratory rate for each individual experiment. Means ± SEM for 4 experiments are given.
*$P < 0.05$ when tested against basal control with a two-tailed *t*-test.
†$P < 0.05$ and ‡$P < 0.01$ when tested against basal control with a paired *t*-test.

the presence of an elevated glucose level reflects high transcriptional rates or changes in insulin mRNA degradation. We have observed that incubation with 5 μg/ml actinomycin D for 24 h failed to affect insulin mRNA levels of rat islets at any of the glucose concentrations tested. This observation suggests slow turnover rates for insulin mRNA and that the rate of insulin mRNA degradation may be a factor of importance for the effects of glucose.[21] There is further evidence in short-term experiments for a heightened insulin mRNA transcription in islets by glucose,[22] although the magnitude of the reported effect barely exceeded the nonspecific effect of glucose on uridine labeling in islets. We recently found a higher rate of insulin mRNA formation than of total RNA synthesis in rat islets in response to glucose.[23] The insulin mRNA content thus appears to be regulated via both transcription and mRNA degradation in the B-cell.

The present observation that islets cultured in the presence of either leucine or 2-ketoisocaproate failed to respond to glucose with an increased insulin release probably reflects a metabolic adaptation at an early glycolytic step.[24,25] However, the preservation of the glucose responsiveness of insulin synthesis in these islets is consistent with the observation that the specific stimulation of insulin biosynthesis becomes saturated at lower concentrations of glucose[17,26] than does that of insulin release.[9] We have nevertheless observed that 2-ketoisocaproate + glutamine stimulate insulin biosynthesis less markedly than glucose, although the effects of these compounds on respiration appear to be more pronounced. It therefore seems that some glycolytic intermediates may be important for stimulation of insulin biosynthesis in addition to the metabolic flux, per se. Indeed, Jackson et al.[27] have recently shown that glucose and its metabolites stimulate protein biosynthesis in a reticulocyte-derived cell-free system.

In conclusion, both insulin biosynthesis and release, and the islet insulin mRNA content correlate roughly with oxidative fluxes in islets (measured as O_2 uptake), suggesting that all of these processes are regulated by metabolic fluxes in the islets rather than by the presence of the glucose molecule per se.

ACKNOWLEDGMENTS

The technical assistance of Ing-Britt Hallgren is gratefully acknowledged. We appreciate the suggestions and critical reviewing of the manuscript by Dr. Donald F. Steiner.

The work was supported by the Swedish Diabetes Association, the Swedish Medical Research Council (12X-109), and the Nordic Insulin Fund.

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