Effect of Glucose on Initiation and Elongation Rates in Isolated Rat Pancreatic Islets*

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SUMMARY

Isolated rat pancreatic islets incubated in media containing glucose at 16.6 mM incorporate [3H]leucine into protein at twice the rate of islets incubated in glucose at 2.8 mM. Increased incorporation was independent of new RNA synthesis, so the effects of glucose on initiation and elongation rates were determined.

The relative rates of elongation at 2.8 and 16.6 mM glucose were determined by pulsing islets in [3H]leucine for 6 min and measuring the ratio of incorporation into soluble (S) and polysome associate (P) peptides. At 2.8 mM glucose, S/P = 3.41 ± 0.28, and at 16.6 mM glucose, S/P = 4.72 ± 0.27, an apparent 28% increase in elongation at the higher glucose. The ratio method assumes that the size of the proteins synthesized is the same. Islets proteins were labeled with [14C]lucine at 16.6 mM glucose and with [3H]leucine at 2.8 mM glucose and subjected to co-electrophoresis on sodium dodecyl sulfate polyacrylamide gels, and the average molecular weight of proteins synthesized at the higher glucose concentration was found to be about 26% smaller. After correction for differences in the size of proteins synthesized, there was no significant effect of glucose on elongation.

Evidence that glucose regulates total islet protein synthesis at the level of initiation includes: (a) increased [3H]leucine incorporation into nascent peptide with the distribution on heavier polysome aggregates indicating an increased number of ribosomes per mRNA, even when new RNA synthesis is blocked; (b) a reduction in the number of ribosomes per mRNA by aurintricarboxylic acid (10−5 M) at 16.6 mM glucose with no significant change at 2.8 mM glucose; (c) when elongation is partially inhibited with low doses of cycloheximide (2 µg per ml), ribosomes accumulate on mRNA at 2.8 mM glucose but not at 16.6 mM glucose; under these conditions the amount of protein synthesized is proportional to the amount of mRNA available for translation. Total islet mRNA appeared to be the same at both glucose concentrations, and the doubling of protein synthesis in the absence of mRNA appeared to be the same at both glucose concentrations. Over-all initiation rate.

While mammalian cells deprived of glucose have diminished rates of protein synthesis (1-4), alterations of glucose concentrations within the physiological range of 2 to 20 mM increase rates of protein synthesis only in the islets of Langerhans of the pancreas (5-10). Previous studies demonstrated that isolated rat islets incorporated about twice as much [3H]leucine into protein when incubated in 15.3 mM glucose compared to 2.8 mM glucose (8-10). This glucose-stimulated increase in incorporation was associated with marked changes in islet RNA metabolism (11) and with an increase in the number of ribosomes active in protein synthesis (i.e. polyribosomes) (12). Regulation could therefore occur at a number of levels, including synthesis of islet mRNA, or at initiation and elongation of peptide synthesis. It was previously demonstrated that glucose activation of islet ribosomes occurs in the presence of actinomycin D at a concentration sufficient to inhibit islet RNA synthesis by 90% (13). Since RNA synthesis was not rate-limiting, this study was undertaken to determine the effects of varying glucose concentrations on initiation and elongation rates in isolated rat pancreatic islets.

EXPERIMENTAL PROCEDURE

Materials—[3H]Leucine (35.5 Ci per mmole) and l-[14C]leucine (311 mCi per mmole) were obtained from New England Nuclear Corporation; sodium deoxycholate, aurintricarboxylic acid, cycloheximide, and sodium dodecyl sulfate from Sigma Chemical Company; t-amino acids from Calbiochem; sucrose (ribonuclease-free) from Schwarz-Mann; crude collagenase from Worthington; all other chemicals were reagent grade from Fisher. NCS tissue solubilizer was from Amersham-Searle.

Islet Preparation and Incubation—Islets of Langerhans were isolated from adult male Sprague-Dawley rats (300 to 450 g) allowed free access to Purina chow diet, by a slight modification of the previously described method (14). Pancreas from two rats

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were minced finely in cold Hanks’ buffer (140 mM NaCl, 6 mM KCl, 1.3 mM CaCl₂, 1.3 mM MgSO₄, 0.3 mM Na₂HPO₄, 0.4 mM KH₂PO₄, and NaHCO₃, 7.5% w/v, added to adjust pH to 7.4), the fat was decanted, then mixed with 50 mg of crude collagenase in 5 ml of Hanks’ buffer, and digested with vigorous stirring with a magnetic stirring bar at 37° for 16 to 20 min. The method was modified by centrifuging the digest at 500 × g for 0.5 min, the collagenase containing supernatant was discarded, and the pellet was vigorously resuspended in fresh buffer by hand shaking. This was repeated four to six times with aliquots observed under the microscope until islets free of acinar tissue predominated. Since islets have a connective tissue capsule which is destroyed on prolonged incubation with collagenase, it was found that this modification gave more reproducible islet yields, about 250 to 500 islets per preparation. Islets were removed with a drawn out capillary pipette, placed into Petri dishes, and washed 10 to 20 times with fresh buffer to minimize nucleae contamination from acinar tissue and collagenase. Islets were incubated in small siliconized Dounce homogenizers in 250 to 500 μl of Krebs’-Ringer bicarbonate-buffered media (107 mM NaCl, 5.9 mM KCl, 1.1 mM MgCl₂, 1 mM CaCl₂, NaHCO₃, 7.5% w/v, added to adjust pH to 7.4; and bovine albumin, 1% w/v) at 37° (unless otherwise stated) in a Dubofshaker at 40 rpm, continually gassed with 5% CO₂-95% O₂ to maintain the pH at 7.4.

**Measurement of elongation rates by this method must be corrected for differences in molecular weights of proteins synthesized under different experimental conditions.** (19) The average molecular weight of proteins synthesized in islets incubated for 45 min in 16.6 mM glucose then pulsed with [3H]leucine incorporated about twice as much label into polysomes labeled for 5 min. On the other hand, when polysomes were labeled for 15 min, puromycin removed significantly less radioactivity, suggesting that the labeled amino acid may be incorporated into ribosomal protein. The radioactivity in the soluble protein increases linearly after a lag (16, 17). This is due to labeling of nascent peptides on polysomes followed by release into soluble proteins. The ratio of released peptides to polysome-associated peptides at 2.8 versus 16.6 μM glucose, is a measure of the relative rate of elongation (18, 19). As seen in Table I, islets incubated for 45 min in 16.6 μM glucose then pulsed with [3H]leucine incorporated about twice as much label into both nascent and soluble peptide as did islets similarly treated but in 2.8 μM glucose. The ratio of incorporation during the 6-min pulse into soluble versus nascent proteins at 16.6 μM glucose was 4.7 compared to a ratio of 3.4 at 2.8 μM glucose. Increased glucose appeared to increase the relative rate of elongation by about 28%.

**Table I**

| Glucose | Soluble | Polyosomal | Ratio of soluble to polyosomal |
|---------|---------|------------|-------------------------------|
| 2.8     | 6,700 ± 1,810 | 1,800 ± 331 | 3.41 ± 0.28 |
| 16.6    | 16,600 ± 1,549 | 3,560 ± 381 | 4.72 ± 0.27 |
| p < 0.01| p < 0.02 | p < 0.02   |                               |

**Preparation and Analysis of Polyribosomes—Polyribosomes are rapidly labeled with a short pulse of radioactive amino acids.** (12) That the label is in nascent peptide was demonstrated by the fact that puromycin removed greater than 90% of the radioactivity from polysomes labeled for 5 min. On the other hand, when polysomes were labeled for 15 min, puromycin removed significantly less radioactivity, suggesting that the labeled amino acid may be incorporated into ribosomal protein. The radioactivity in the soluble protein increases linearly after a lag (16, 17). This is due to labeling of nascent peptides on polysomes followed by release into soluble proteins. The ratio of released peptides to polysome-associated peptides at 2.8 versus 16.6 μM glucose, is a measure of the relative rate of elongation (18, 19). As seen in Table I, islets incubated for 45 min in 16.6 μM glucose then pulsed with [3H]leucine incorporated about twice as much label into both nascent and soluble peptide as did islets similarly treated but in 2.8 μM glucose. The ratio of incorporation during the 6-min pulse into soluble versus nascent proteins at 16.6 μM glucose was 4.7 compared to a ratio of 3.4 at 2.8 μM glucose. Increased glucose appeared to increase the relative rate of elongation by about 28%.

Measurement of elongation rates by this method must be corrected for differences in molecular weights of proteins synthesized under different experimental conditions. (19) The average molecular weight of proteins synthesized at 16.6 μM glucose was compared to that at 2.8 μM glucose by incubation of islets in [14C]leucine and [3H]leucine, respectively. Labeled

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1 M. A. Permutt, unpublished observation.
proteins then were solubilized and electrophoresed on sodium dodecyl sulfate polyacrylamide gels where the mobility on the gels is proportional to the log of the molecular weight. Islet proteins synthesized in 16.6 mM glucose have a different molecular weight profile on sodium dodecyl sulfate polyacrylamide gel electrophoresis than proteins synthesized in 2.8 mM glucose (Fig. 1). The over-all pattern of proteins synthesized appeared to be qualitatively similar at both glucose concentrations, but there was a relative increase in synthesis of lower molecular weight peptides at 16.6 mM glucose. The average molecular weight of proteins synthesized at 16.6 mM glucose was 34,000, compared to 46,000 at 2.8 mM glucose (average of three gels each). When the apparent increased elongation rate (28%) at 16.6 mM glucose is corrected (19) for synthesis of peptides which are on the average 26% smaller, the relative rates of elongation are virtually the same. A change in the elongation rate, therefore, is not the explanation for the doubling in incorporation seen in Table I.

Polypeptide Initiation—A number of observations suggest that the effect of glucose on islet protein synthesis is through increased initiation of translatable mRNA. It was previously demonstrated that the increased protein synthesis at 16.6 mM glucose is associated with an increase in [3H]leucine incorporation into nascent peptide which corresponds to the number of ribosomes active in protein synthesis (Ref. 13; see also Table I) and that this increase was independent of new RNA synthesis. The distribution of nascent peptide on islet polysomes was also shifted to heavier polysome aggregates, suggesting that at 16.6 mM glucose the average mRNA is loaded with more ribosomes than at 2.8 mM glucose.

Further evidence that initiation is rate limiting at 2.8 mM glucose was obtained by incubating islets in aurintricarboxylic acid (ATA), an antibiotic which inhibits initiation in mammalian cells (20). ATA at 10^{-5} M produced about a 50% reduction in protein synthesis at 16.6 mM glucose associated with a decrease in [3H]leucine incorporation into nascent peptide (Fig. 2A). ATA also shifted the distribution of nascent peptide to smaller polysomes. ATA at 10^{-5} M had negligible effects on protein synthesis in islets incubated in 2.8 mM glucose (Fig. 2B).

Islet mRNA: Amount and Rates of Utilization—Several investigators have recently been able to estimate the amount of mRNA and its utilization rates under various experimental conditions by partially inhibiting elongation (6, 21, 25). Since the amount of protein synthesized over a period of time (sufficiently greater than the translation time) is proportional to the amount of mRNA and the initiation of the messenger, when elongation is slowed ribosomes build up, and the amount of protein synthesized is no longer limited by initiation. If the mRNAs are saturated with ribosomes, the amount of protein synthesized is proportional to the amount of available mRNA. Low doses of cycloheximide were used to partially inhibit elongation of islet protein synthesis which was associated with a buildup of ribosomes on mRNA in islets incubated at 2.8 mM glucose, but not at 16.6 mM glucose (Fig. 3), which is further evidence that initiation is rate-limiting at low glucose.

Partial inhibition of elongation with low dose cycloheximide

* The abbreviation used is: ATA, aurintricarboxylic acid.
was performed in isolated islets to determine the effects of glucose on the mRNA activity for total islet proteins, and specifically for proinsulin and insulin. Islets incubated in 16.6 mM glucose incorporated about as much [3H]leucine into total protein as did islets in 2.8 mM glucose (Table II). Incorporation of [3H]leucine at both concentrations of glucose in the presence of low dose cycloheximide (2 μg per ml) was not significantly different. This observation suggests that the amount of mRNA available for translation of total islet proteins is the same at low and high glucose, and the difference in incorporation is due to different rates of translation of the mRNA. Since the over-all rate of elongation is the same, the doubling of protein synthesis in high glucose is most likely due to a doubling of the over-all rate of initiation.

The [3H]leucine-labeled islet proteins synthesized at 2.8 and 16.6 mM glucose ± low dose cycloheximide were electrophoresed on sodium dodecyl sulfate polyacrylamide gels, and an estimate of the rate of translation of individual islet messengers was obtained (Fig. 4). Non-globin proteins in reticulocytes (22), and adenovirus infected HeLa cells (23) have been similarly studied but, unfortunately, islet proteins are heterogeneous and cannot be clearly resolved into discrete protein bands. Nevertheless, if it is assumed that the rate of translation of individual proteins is the same in low dose cycloheximide, it appears that the amount of mRNA present for larger molecular weight proteins (Fig. 4C) is as abundant as for smaller proteins, but the increased synthesis of smaller proteins at 16.6 mM glucose is due to increased utilization of smaller messengers (Fig. 4D). Even at 2.8 mM glucose there is evidence for differential rates of messenger utilization (Fig. 4, compare A to B).

The experiment demonstrates that the effect of glucose on proinsulin-insulin synthesis is more complex than the effect on total islet protein synthesis. As noted previously (10), the glucose stimulatory effect is much greater for proinsulin-insulin synthesis, so that proinsulin and insulin account for about 2% of total islet protein synthesis at 2.8 mM glucose and for 18% at 16.6 mM glucose. When elongation was partially inhibited, there was still a significant 3-fold difference in proinsulin-insulin synthesis. This suggests that, in contrast to total mRNA activity, mRNA available for translation of proinsulin is increased 3-fold by high glucose. Since mRNA activity accounts for only part of the glucose stimulatory effect, the remainder must be due to increased translation of mRNA for proinsulin.

The effect of glucose on the relative elongation rate for proinsulin has not been determined, and it is therefore not possible to say whether the translational effect is due to increased initiation, elongation, or both.

**DISCUSSION**

The purpose of this study was to determine how alterations of glucose in the media, within the physiological range of 2 to 20 mM, affect the rate of protein synthesis in isolated islets. A number of levels of regulation could exist, including the synthesis of islet mRNA, activation of pre-existing mRNA, increased rate of initiation, or increased elongation rates. Previous studies with actinomycin D showed that glucose stimulated islet protein synthesis was associated with polysome aggregation which was independent of new RNA synthesis (13). An increase in the number of translatable messengers which were previously synthesized, however, remains a possibility (24). This seems unlikely because an increase in the number of available mRNAs without a change in the rate of initiation would increase nascent peptide formation but not produce the shift to synthesis on larger polysome aggregates (25). The experiment where elongation was partially inhibited with cycloheximide (2 μg per ml) also supports the hypothesis that glucose control of total islet protein synthesis is post-transcriptional. With partial inhibition of elongation, the messengers become saturated with ribosomes and the amount of protein synthesized, although reduced, should be proportional to the amount of messenger available for translation. Under these conditions the incorporation of [3H]leucine into total islet protein was not significantly different at 2.8 or 16.6 mM glucose (Table II).

Measurement of relative rates of elongation in tissues using the ratio method has been accomplished in tissue culture cells at various temperatures (18) and undergoing mitosis (16) and in the chick oviduct which is estrogen-primed (19). The movement of radioactive material from nascent peptide to soluble chains, the average transit time, is defined by Fan and Penman (16) as the length of time required for a ribosome, after attachment to mRNA, to complete and release the nascent peptide. This average transit time is independent of initiation. Palmiter...
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similar. When islet polysomes are homogenized with detergents,
tional in the tissues studied for the duration of labeling time;
and the molecular weights of the proteins synthesized should be similar.
When altered in glucose had no effect on total islet mRNA
activity or on elongation, the rate limiting event at 2.8 mm
caused to appear to be initiation. Evidence supporting this hy-
thesis includes: (a) increased nascent peptide formation on larger polysome aggregates in 16.6 mm glucose indicated an
increased number of ribosomes per messenger (13); (b) partial
inhibition of initiation by ATP in 16.6 mm glucose decreased the
number of ribosomes per messenger, but not in 2.8 mm glu-
ose; and (c) loading of ribosomes on mRNA in 2.8 mm glucose
when elongation was partially blocked with low doses of cyclo-
ximide, which did not occur in 16.6 mm glucose.
Altogether, glucose in the incubation media have a specific
change in proinsulin-insulin biosynthesis relative to total islet
protein as proinsulin-insulin accounts for about 2% at 2.8 mm
and 18% at 16.6 mm glucose (Table II). Previous
(10) listed several criteria which should be satisfied before this
method can be interpretable, including: the recovery of nascent
and soluble chains should be complete; labeling time should be
sufficient to chase all cold amino acids from nascent peptide, i.e.
at least one round of translation; polysomes should be unde-
graded, the rate of amino acid incorporation should be propor-
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