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Regulation of Proinsulin Synthesis in Isolated Rat Islets (39134)[1]

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The modulation of insulin secretion has received extensive study since the pioneering work of Anderson and Long (1). A number of physiologic and pharmacologic agents have been shown to either stimulate or depress the release of insulin from the islet B-cell (2). More recently, the control of proinsulin synthesis and its conversion to insulin has been a subject of investigation. Glucose (3–5) and other sugars (5), tolbutamide (4, 6, 7), and hormones (8, 9) have been found to alter the rate of insulin production via proinsulin. The most potent agent, namely, glucose, selectively stimulates proinsulin synthesis and probably acts both at transcriptional and post-transcriptional levels (10).

The present report describes an *in vitro* system for evaluating short-term effects of various agents on proinsulin synthesis in the absence of appreciable conversion of new proinsulin to insulin. Of the agents we tested, glucose is the most potent stimulator of proinsulin synthesis and it shows its effect within a period of 30 min. Glucagon and cyclic AMP are also stimulatory, while preliminary studies with pyridine nucleotides show no effect (NADP), or general inhibition (NADPH), of islet protein synthesis.

Materials and methods. Islet isolation and incubation. Male Holtzmann rats weighing 350–500 g were obtained from the Holtzmann Rat Co., Madison, Wisconsin. The animals were anesthetized with sodium diabutol, and islets of Langerhans were isolated from the pancreas by the method of Lacy and Kostianovsky (11) as modified by Lindall *et al.* (12).

The isolated islets were resuspended in 300 μl of incubation medium, which contained 0.154 *M* Krebs bicarbonate solution, common amino acids except leucine (20 mg of each per liter), pancreatic trypsin inhibitor (PISF) (at 1 mg/liter), bovine serum albumin (200 mg/liter), and the experimental agents. Following 60 min of incubation at 37° in a shaking bath under oxygen–carbon dioxide (95:5), the postincubation medium (200 μl of incubation medium plus 100 μCi of [4,5-^{3}H]L-leucine) was added, for an additional 30 min of incubation under the same conditions. Using this procedure, the islets were exposed to experimental agents for a total of 90 min, but to the radioactive leucine precursor only for sufficient time for proinsulin synthesis to occur, and without significant conversion of proinsulin to insulin.

Assay of proinsulin synthesis. The islets were homogenized in 1 ml of water containing carrier BSA (1 mg) and nonlabeled leucine, and extracted by a procedure modified from Davoren (14) and Kenny (15). Proteins of the final extract were separated by gel filtration (Sephadex G-50 fine) in 1 *M* acetic acid. The highest incorporation of radioactivity occurred between the void volume and insulin. That this peak represents [^{3}H]proinsulin is indicated by trypsin treatment which cleaves essentially all of this material to a peptide migrating with the insulin marker. The absence of significant [^{3}H]insulin or single-chain [^{3}H]proinsulin is shown by polyacrylamide gel electrophoresis (Fig. 1). The recovery of [^{3}H]proinsulin by gel electrohoresis did not compare favorably with gel filtration, and for this reason, gel filtration was the method of choice in establishing this system.

Samples were assayed for radioactivity by liquid scintillation spectrometry for sufficient time to ensure errors under 5%, at a

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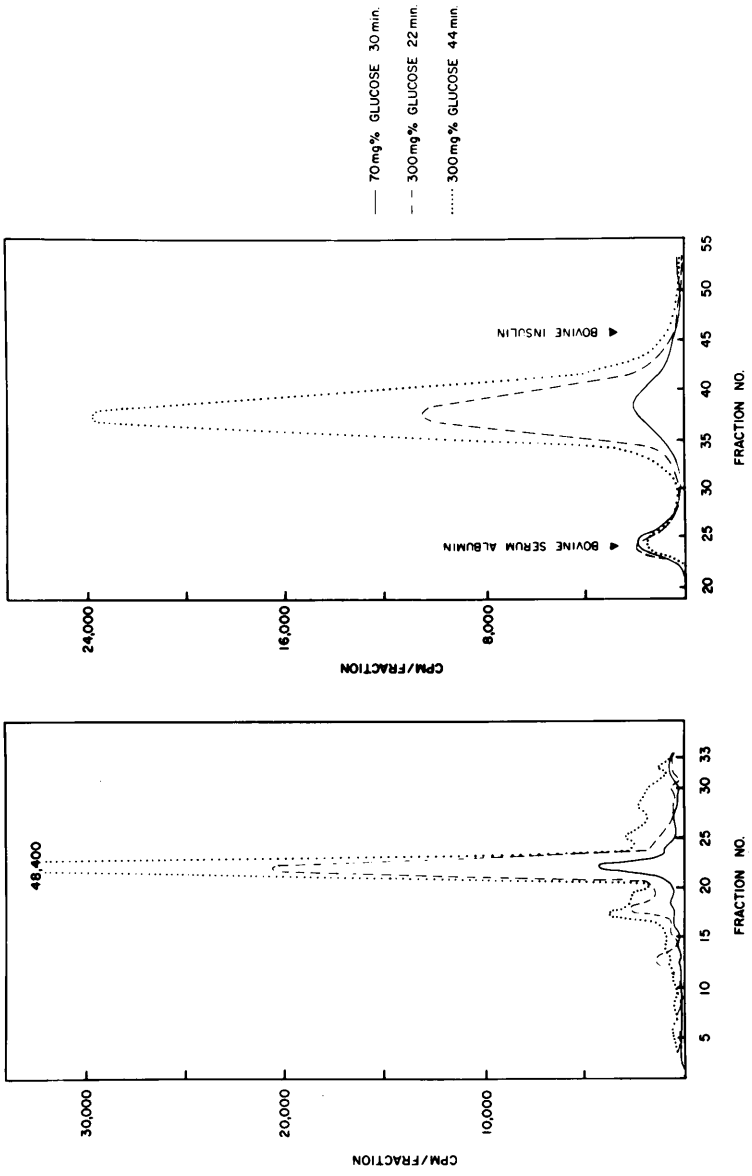

FIG. 1. Recovery of [^{3}H]proinsulin by gel electrophoresis versus gel filtration. Isolated rat islets were incubated under conditions indicated in the legend. The final extracts were divided in half and subjected to either separation procedure.

24% efficiency for tritium. To estimate the total radioactivity associated with proinsulin, the sum of background-corrected counts was calculated in fractions from the trough to the left of the proinsulin peak, to the trailing edge of the proinsulin peak. Comparison of control and test incubations was done using the "paired comparison" *t* test.

Polyacrylamide gel electrohoresis (PAGE) on 7% polyacrylamide (pH 8.3) was carried out using the Canalco (Bethesda, Md.) Model 6 apparatus. Insulinlike activity of the islets was determined by the double antibody method of Morgan and Lazarow (16) using rat insulin standards.

Results. Viability of isolated islets. Islets of Langerhans isolated by our procedure were viable on the basis of amino acid incorporation into protein and response to glucose. The protein content of islets was determined by the method of Lowry *et al.* (13) using bovine albumin for standards and was found to be 0.99 ± 0.04 μg/islet (eight determinations); the immunoassayable insulin content, using rat insulin standards, was 1.68 ± 0.15 munits/islet (eight determinations).

When porcine [^{131}I]insulin was added as a tracer to 30 islets processed by the extraction procedure, 87% of the total radioactivity was recovered in the final extract (FE). In a typical incubation (in medium having a glucose concentration of 100 mg%) 96.8% of [^{3}H]leucine radioactivity remained soluble. Of the remaining radioactivity, 82.9% was incorporated into Bulk Protein (trichloroacetic acid residue after acid alcohol extraction) and 17.2% into the FE.

In order to determine whether islets were capable of sustained leucine incorporation into proinsulin, islets were incubated in the presence of 300 mg% glucose for 60 min, and then postincubated for 20, 30 (routine), 40, and 60 min with addition of [^{3}H]leucine (Fig. 2). Since incorporation into proinsulin was essentially linear for extended periods, under a highly stimulatory glucose concentration, the islets were judged satisfactory for these studies. Cycloheximide (100 μg/ml) inhibited leucine incorporation into proinsulin to the extent of 90% in this system.

Glucose effect on protein synthesis. To obtain an estimate of the stimulatory effect of glucose on proinsulin synthesis in our system, islets were exposed either to control (70 mg%) or high (300 mg%) glucose during 90 min of incubation. As seen in Table I, the rate of proinsulin synthesis was increased to over five times the control level. When islets were exposed for only 30 min to either of the two glucose levels,

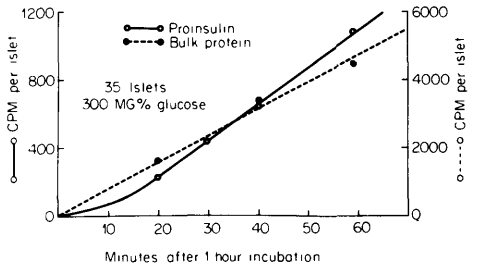

FIG. 2. Time course of [^{3}H]leucine incorporation into rat islet proteins. Isolated islets were incubated for 60 min in incubation medium containing 300 mg% glucose, and postincubated for the specified times in the same medium containing [^{3}H]leucine.

TABLE I. COMPARISON OF THE EFFECTS OF HIGH GLUCOSE CONCENTRATION FOR 30 MIN AND 90 MIN ON [^{3}H]LEUCINE INCORPORATION INTO RAT PROINSULIN.

Experiment	Specific activity leucine (Ci/mmole)	Control[a] CPM/islet	Experimental CPM/islet	Difference (%)
A. High glucose (300 mg%) during incubation (60 min) and postincubation (30 min)				
1	5	58	481	+730
2	5	48	256	+434
3	58.2	161	946	+487
Mean difference from control (%) SEM				+556 ±91
				$P < 0.05$
B. Low glucose (70 mg%) during incubation (60 min) and high glucose (300 mg%) during postincubation (30 min)				
1	5	76	105	+38.0
2	5	73	100	+37.5
3	5	41	55	+33.5
Mean difference from control (%) SEM				+36.3 ±1.2
				$P < 0.01$

[a] 70 mg% throughout incubation and postincubation.

proinsulin synthesis was found to be significantly higher in 300 mg% glucose than in 70 mg%.

Glucagon and cyclic AMP effects on proinsulin synthesis. Since glucagon and cyclic AMP have been reported to stimulate insulin secretion, it was of interest to determine their effects on proinsulin synthesis. They are considered together because of their possible influence on the islet adenyl cyclase system. Using a 60-min incubation and 30-min postincubation with [^{3}H]leucine, both glucagon (10 μg/ml) and cyclic AMP (7.6 m*M*) showed modest, but significant, stimulation of leucine incorporation into proinsulin at 70 mg% glucose (Fig. 3). At a glucose level of 300 mg%, these effects were less marked. Caffeine (1.3 m*M*), which inhibits phosphodiesterase and therefore may increase endogenous islet cyclic AMP levels, also stimulated proinsulin synthesis.

Effects of NADPH and NADP on proinsulin synthesis. NADPH has been reported to stimulate insulin release from islet tissue (17, 18) and from isolated insulin secretion granule fractions (19). At concentrations of 10^{-2} and 10^{-3} *M*, NADPH significantly inhibited proinsulin synthesis (at 70 mg% glucose) while NADP had no effects at any concentrations used. Raising the glucose level to 300 mg% lowered the inhibition by NADPH (Fig. 4). That NADPH inhibits islet protein synthesis in general is shown by its action on the Bulk Protein fraction (Fig. 5).

Discussion. Rat Islets of Langerhans, isolated by collagenase digestion, secrete insu-

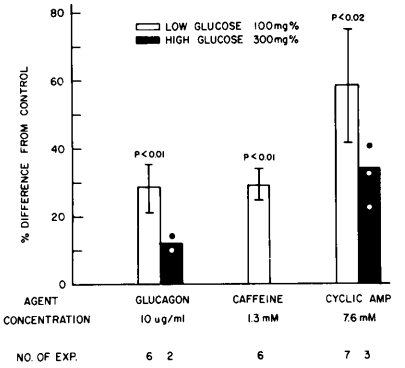

FIG. 3. Summary of effects of glucagon, caffeine, and cyclic AMP on [^{3}H]leucine incorporation into rat proinsulin. Incubation conditions are detailed under Materials and methods.

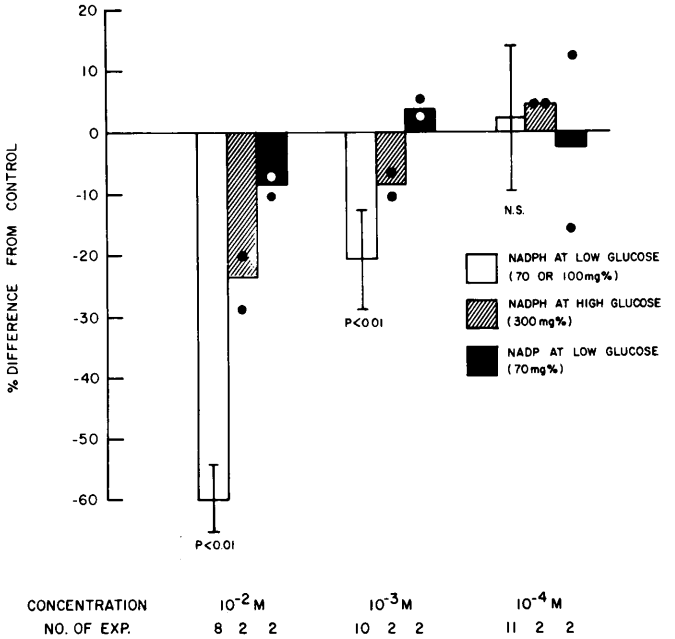

FIG. 4. Summary of effects of pyridine nucleotide additions on [^{3}H]leucine incorporation into rat proinsulin. Routine incubation conditions.

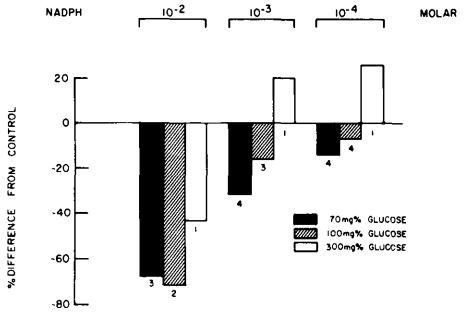

FIG. 5. Effects of NADPH on [^{3}H]leucine incorporation into Bulk Protein. NADP had no effect on this parameter (data not shown).

lin in response to glucose, cyclic AMP, glucagon, caffeine, and reduced pyridine nucleotides (18, 20). In the present study, a system was devised to evaluate the early effects of these and other agents on the rate of proinsulin synthesis in rat islets. After 90 min of *in vitro* incubation (30 min in the presence of precursor [^{3}H]leucine) each of the test agents mentioned above showed statistically significant effects on proinsulin synthesis. No significant conversion of radioactive proinsulin to intermediates or insulin was observed by gel filtration or gel electrophoresis of the extracted proteins. Little or no radioactive protein release or secretion would be expected during the brief (30-min) exposure to [^{3}H]leucine, thus ensuring the complete recovery of radioactive proteins in the tissue extract. In long-term incorporation studies, the conversion of proinsulin to intermediates and insulin (plus connecting peptide) changes the apparent rate of proinsulin synthesis. This may be corrected for by combining the radioactivity in all of these proteins (neglecting losses of connecting peptide) or as shown in this study, the cleavage may be prevented by choosing the appropriate incubation conditions.

In the present study, [^{3}H]proinsulin synthesis was estimated in extracts by means of gel filtration, since recovery of [^{3}H]proinsulin was found to be superior to that obtained by polyacrylamide gel electrophoresis (PAGE). However, assuming the loss of [^{3}H]proinsulin to be constant, PAGE would be useful in further work, since it allows many simultaneous separations.

As shown here and elsewhere (10), the addition of glucose at concentrations above 70 mg% stimulated the synthesis of [^{3}H]proinsulin within a 30-min period of incubation, and this effect is marked at 90 min of incubation. The rate of proinsulin synthesis lags initially, confirming a previous observation (10), but subsequently achieves linearity over a 2-hr period under maximal stimulation by glucose. Under the same conditions, Bulk Protein synthesis proceeds linearly throughout the incubation period (Fig. 2).

Evidence for participation of the adenyl cyclase system in proinsulin synthesis has been reported previously (8, 21). In the present study, cyclic-3′,5′-AMP (7.6 m*M*) stimulated proinsulin synthesis by about 60% at normal glucose concentrations (70–100 mg%). The addition of caffeine or glucagon also stimulated proinsulin synthesis, suggesting that the concentration of intracellular cyclic AMP regulates the rate of proinsulin synthesis. However, exogenous cyclic AMP was more effective in eliciting a response than either glucagon or caffeine, both of which presumably act by increasing endogenous cyclic AMP concentrations. This might be a dose-dependent effect, as we have not yet determined the appropriate concentrations of caffeine and glucagon necessary for maximal stimulation. On the other hand, exogenous and endogenous cyclic AMP may have different modes of action.

The pyridine nucleotide NADPH has been shown to stimulate insulin secretion in fish (17) and mammalian islets (18). Its action in this respect is interesting in that it also causes release of immunoassayable insulin from isolated storage granules (19). When added in our system at concentrations that are known to stimulate insulin release, NADPH markedly inhibited proinsulin and Bulk Protein synthesis; NADP had no effect on these parameters. The effect of NADPH was less pronounced at 300 mg% glucose than at 70–100 mg%, which suggests that the high glucose concentration in the medium protected against the action of this agent.

The dual action of NADPH on insulin secretion and synthesis are reminiscent of

similar effect of sulfonylureas (4, 6, 7), and provide more evidence for the theory that the rates of insulin secretion and synthesis are uncoupled (cf. 21). Whether changes in levels of NADPH within islet tissue influence the physiologic regulation of insulin biosynthesis and secretion remains to be determined.

Summary. (1) A system is described for studying the short-term effects of agents on proinsulin synthesis *in vitro*, as measured by the incorporation of [^{3}H]leucine into isolated proinsulin. (2) Of the agents tested, glucose has the most marked, and apparently earliest, effect on proinsulin synthesis. (3) The adenyl cyclase system participates in the regulation of proinsulin synthesis since exogenous cyclic AMP, glucagon, and caffeine are stimulatory. When cyclic AMP is added to the medium in the presence of glucose, it is the most potent agent acting on the adenyl cyclase–phosphodiesterase system. (4) The addition of NADPH to isolated rat islets inhibits proinsulin and Bulk Protein synthesis *in vitro*.

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