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Research Article

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Functional Differences between Rat Islets of Ventral and Dorsal Pancreatic Origin

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ABSTRACT Do functional linkages between islet endocrine cells exist? The effect of differences in frequency and distribution of islet endocrine cells on B cell function was examined in islets from the ventral (ventral islets) and dorsal (dorsal islets) areas of the rat pancreas.

Dorsal islets contained 10 times as much glucagon as ventral islets, whereas insulin and total protein contents were similar. Basal rates of insulin secretion and proinsulin biosynthesis were similar in the two types of islet, but, under conditions of glucose stimulation, both insulin secretion and proinsulin biosynthesis were significantly greater in the glucagon-rich dorsal islets. Similarly, glucose utilization rates and ATP levels were greater in dorsal islets. In contrast, the rates of processing of newly synthesized proinsulin were similar in ventral and dorsal islets. That the islet glucagon content may have affected B cell function is inferred from two independent findings. Firstly, basal and glucose-stimulated cyclic AMP contents of glucagon-rich dorsal islets were greater than those of ventral islets. Secondly, in the presence of excess exogenous glucagon (1 $\mu\text{g}/\text{ml}$), the differences in glucose-induced insulin secretion and proinsulin biosynthesis rates between the two types of islets were eliminated.

These results strongly suggest that changes in the relative proportions of the different islet endocrine cells exert marked effects on islet function. In particular, a greater A cell and glucagon content is associated with higher rates of glucose-induced insulin secretion and biosynthesis.

INTRODUCTION

In man and other species it has been established that the endocrine cell composition and distribution in pancreatic islets are different in different parts of the

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pancreas and that in any one species the cellular composition is relatively fixed within a given pancreatic region. In the rat, for example, islets in the lower two-thirds of the pancreatic head (ventral area) differ in cellular composition from those in the remainder of the pancreas (dorsal area). Islets in the ventral area (ventral islets) and in the dorsal area (dorsal islets) have a central core of insulin-containing B cells that constitutes a similar proportion of the total endocrine cells in both islet types (1). The peripheral area of the islet contains pancreatic polypeptide-, glucagon-, and somatostatin-containing cells. In ventral islets pancreatic polypeptide-containing PP cells constitute a much greater percentage of the total endocrine cell number than glucagon-containing A cells; the opposite is the case in dorsal islets (1). The somatostatin-containing D cell number is within the same order of magnitude for the two islet types. The nonrandom arrangement of cells within the islet has suggested that the endocrine cells are functionally interrelated (2).

In view of the known effects of circulating insulin, glucagon, and somatostatin on the secretion of each of these hormones (see reference 3 for a review), it seems reasonable to postulate that pancreatic B cell activity in ventral and dorsal islets of the rat might be influenced by differences in the local concentrations of hormones secreted by the other endocrine cells and/or by direct communications between neighboring endocrine cells (4-7).

We (8) have shown that glucose-stimulated insulin secretion by glucagon-rich islets from the dorsal region of the rat pancreas is greater than that by ventral islets, although the insulin contents of the dorsal and ventral islets were similar. In the studies presented in the present paper we have confirmed our previous findings and have extended the scope to include investigations of proinsulin biosynthesis and proinsulin processing in the two islet types. In addition, we have compared the glucose utilization rates and ATP and cyclic AMP contents of the islets under various experimental condi-

tions. The possibility that some of the observed functional differences between the ventral and dorsal islets may be related to differences in their glucagon content is discussed.

METHODS

Pancreatic islets were isolated by a modification of the collagenase technique (9) from ad lib.-fed male Wistar rats (190–260 g). Eight rats were used for each experiment. Previous experiments had shown that the glucagon-poor part of the rat pancreas occupies the lower two-thirds of the head and that the glucagon-rich area comprises the upper one-third of the head as well as the body and tail of the pancreas. The lower one-third or two-fifths of the pancreatic head (ventral area) was dissected carefully from each rat. Similar amounts of tissue were taken from the dorsal area of the same pancreata and were treated simultaneously but separately with collagenase (Serva GmbH, Heidelberg, West Germany). Care was taken to keep tissue volume and collagenase concentration similar for the digestion of tissue from the two different pancreatic regions, and the time taken for digestion was the same. Care also was taken to choose islets of the same size from the two digestates. In all experiments islets were first preincubated for 30 min at 37°C in modified Krebs-Ringer-Hepes buffer (KRB-Hepes)¹, containing 10 mM Hepes (Gibco Bio-Cult, Glasgow, Scotland), 5 mM NaHCO₃, 1 mM CaCl₂, and 250 KIU/ml aprotinin (Trasyol; provided by Prof. G. L. Haberland, Bayer AG, Wuppertal, West Germany), and 5 mg/ml bovine serum albumin (BSA; fraction V, Behringwerke AG, Marburg/Lahn, West Germany), pH 7.4. The KRB-Hepes used for the preincubations contained 2.8 mM glucose. (The only exception to the buffer described above was used in experiments for determination of islet protein content; see below.) After preincubation, islets were washed twice in fresh KRB-Hepes containing 2.8 mM glucose and distributed in groups of 10 into glass incubation vials with 1 ml KRB-Hepes or 1 ml Dulbecco's modified Eagle's medium (DMEM) that contained no unlabeled leucine (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.) and 0.5 g/100 ml BSA. In the investigation of proinsulin processing, 40 islets were incubated in 4 ml of KRB-Hepes.

Insulin secretion and proinsulin biosynthesis. Islets were incubated for 30 min at 37°C in the continuous presence of the test substances and 100 µCi/ml L-[4,5-³H]leucine (Radiochemical Centre, Amersham, Bucks, England). The specific radioactivity of the leucine was 72 Ci/mmol. The test substances were glucose, glucagon (Novo Industri SA, Bagsvaerd, Denmark), and 3-isobutyl-1-methylxanthine (IBMX) (Sigma Chemical Co. St. Louis, Mo.). At the end of the 30-min incubation the islets and media were rapidly cooled to 4°C, and the medium was removed and stored at -20°C until assayed for insulin. The islets were then washed three times in KRB-Hepes containing 2.8 mM glucose. After the third wash all KRB-Hepes was removed, and 1 ml of 0.2 M glycine containing 2.5 mg/ml BSA, pH 8.8 (glycine-BSA)

was added to each tube containing 10 islets. The islets were sonicated and centrifuged (30,000 g for 30 min at 4°C) to remove cell debris. No loss of immunoreactive insulin was detectable after sonication and ultracentrifugation by this method. The supernatants from this centrifugation were used for determination of incorporation of [³H]leucine into proinsulin. To determine the amount of radioactivity incorporated into proinsulin, aliquots of the supernatants from the islet sonicates were subjected to quantitative immunoprecipitation as described in detail elsewhere (10). In brief, aliquots of islet sonicates were incubated at room temperature for 1 h in the presence of a 50- to 100-fold excess (binding capacity relative to total immunoreactive material present in sample) of anti-insulin serum (Miles-Yeda, Rehovot, Israel). The total incubation volume was 200 µl of 0.2 M glycine-BSA, 0.5 g/100 ml (vol/vol) NP-40 (Nonidet P-40, Fluka AG, Buchs, Switzerland). Antigen-antibody complexes were then precipitated by adding 5 mg of protein A-Sepharose (Pharmacia, Zurich, Switzerland) in 100 µl of glycine-BSA, NP-40. After careful mixing for 15 min at room temperature, the immunoreactive material bound to the protein A-Sepharose was separated from unbound material in the supernatant by centrifugation at 8,000 g for 30 s. The precipitate was then washed twice with 250 µl glycine-BSA, NP-40. 250 µl of 1 M acetic acid, 2.5 mg/ml BSA was then added to the pellet to liberate the bound proinsulin and insulin. The supernatant (and washes) and the acetic acid mixture (including the Sepharose) were placed in separate liquid scintillation vials, and the radioactivity was determined in a liquid scintillation counter using 10 ml Biofluor (New England Nuclear, Dreieich, West Germany) as the scintillant. Nonspecific binding was assessed by the use of nonimmune serum instead of anti-insulin serum (1–3% of all radioactive counts present were nonspecifically bound). For each sample, both the binding with anti-insulin serum and the nonspecific binding were assessed in duplicate. All results are expressed as the specifically immunoprecipitable radioactivity (total less nonspecific). By this method, radioactively labeled immunoprecipitable material was found to be quantitatively precipitated and recovered. As a result of the large excess of anti-insulin serum used, both proinsulin and insulin were precipitated quantitatively. (When specifically immunoprecipitated material was subjected to Sephadex G-50 column chromatography, it was found that after a 30-min incubation in the presence of [³H]leucine, although radioactivity was found in the proinsulin position, none of the newly synthesized proinsulin had been processed to insulin. Results not shown.) Insulin and glucagon measurements were made on islet sonicates to determine the total insulin and glucagon content of the islets (see below).

Processing of newly synthesized proinsulin. Islets were incubated in groups of 300–400 for 15 min in 3.0 ml of DMEM that contained no unlabeled leucine, and which was supplemented with 100 µCi/ml [³H]leucine (72 Ci/mmol), 0.5 g/100 ml BSA, and that contained 16.7 mM glucose. These conditions were used to maximize [³H]leucine incorporation into proinsulin during the 15-min labeling period. The medium was then removed, and the islets were washed carefully three times with cold (4°C) KRB-Hepes. Groups of 40 islets were then either measured for total radioactivity in proinsulin or dispensed into vials with 4 ml of KRB-Hepes containing various test substances for a 30-min chase period. At the end of the chase period the buffer was removed, and the islets were washed three times with cold KRB-Hepes. Finally, the islets were suspended in 0.8 ml glycine-BSA, sonicated, and centrifuged (30,000 g for 30 min at 4°C).

¹ Abbreviations used in this paper: BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; glycine-BSA, 1 ml of 0.2 M glycine containing 2.5 mg/ml BSA, pH 8.8; IBMX, 3-isobutyl-1-methylxanthine; KRB-Hepes, modified Krebs-Ringer-Hepes buffer.

Islets not subjected to a chase period were similarly washed, sonicated, and centrifuged. The islet sonicates were then subjected to two independent immunoprecipitations. One was performed as described for the biosynthesis experiments to obtain exact quantitation of [³H]leucine specifically incorporated into proinsulin and insulin. The other immunoprecipitation was carried out with anti-insulin serum and under the same experimental conditions as the first immunoprecipitation, except that when the labeled material was displaced from the antibody-protein A-Sepharose complex by the addition of 1 M acetic acid, 2.5 mg/ml BSA, the solubilized material was applied to a Sephadex G-50 column (0.8 × 70 cm) to separate proinsulin from insulin. The column was eluted with 1 M acetic acid, 2.5 mg/ml BSA (1.2-ml fraction) to prevent rebinding of antigen to antibodies during the chromatography. The amounts of radioactivity appearing in the proinsulin and insulin peaks were then calculated. The amount of labeled proinsulin remaining after the chase period was then compared with that found immediately after the 15-min labeling period (when all specifically immunoprecipitated radioactivity was found in proinsulin; the amount of radioactivity in proinsulin at the end of the labeling period was counted as 100%).

Measurement of islet cyclic AMP content. After a 30-min preincubation (see above), groups of 10 islets were incubated for 2 min in KRB-Hepes buffer at 37°C in glass tubes. Insulin release was measured in an aliquot of the buffer. The remaining buffer was rapidly removed, and the islets were boiled in 0.05 M acetate buffer (pH 6.2). Islet cyclic AMP levels were determined by radioimmunoassay using a commercially available kit (Becton Dickinson, Basel, Switzerland), after succinylation of samples and standards according to the method of Cailla et al. (11).

Measurement of islet ATP content. Islet ATP content was measured by the luciferase method of Stanley and Williams (12) using firefly extract (Worthington Biochemical Corp., Freehold, N. J.).

Measurement of glucose utilization. After a 30-min preincubation in KRB-Hepes containing 2.8 mM glucose, glucose utilization in islets was determined by measuring the conversion of [5-³H]glucose (New England Nuclear) to ³H₂O. The technique employed was similar to that described by Verspohl et al. (13). It measured glucose utilization by way of glycolysis in groups of 10 islets in 1 ml of medium during a 90-min incubation. The islet concentrations used by Verspohl et al. (5 islets/ml) and that of this study (10 islets/ml) are much lower than those most often used to assess glucose utilization in pancreatic islets (see Zawlich and Matschinsky [14] for a review). For the present experiments we felt that a low islet concentration was essential to avoid the build-up of extremely high levels of secreted hormones in the incubation medium, which at least theoretically, could modify the metabolism of the two types of islet in different ways.

Measurement of protein content of islets. Islets in which protein content was measured according to Lowry et al. (15) were washed only with KRB-Hepes to which no albumin had been added.

Hormone assays. Insulin and glucagon were measured by radioimmunoassay by the charcoal separation technique (16). Rat insulin (Novo Research Institute, Bagsvaerd, Denmark) and guinea pig anti-pork insulin serum (Dr. H. H. Schöne, Farbwerke Hoechst, Frankfurt) were used in the insulin assay. Pork glucagon (Novo Research Institute) was used as standard for the glucagon assay. The glucagon antiserum (BR124), used at a final dilution of 1:210,000, was raised in rabbits in our laboratory to pork glucagon coupled

to albumin by difluorodinitrobenzene (17). The antiserum reacted with a C terminal-containing (residues 18–29) but not with N terminal-containing (residues 1–18) fragments of pancreatic glucagon. It showed no cross-reactivity with glicentin, gastric inhibitory polypeptide, gastrin, motilin, cholecystokinin, secretin, vasoactive intestinal polypeptide, somatostatin, pancreatic polypeptide, or insulin.

Results are expressed as mean ± SEM. Student's two-tailed *t* test was used for comparing groups.

RESULTS

Islet content of insulin, glucagon, and protein

A random sample of islets was taken from the various experiments, and their insulin, glucagon, and protein contents were measured.

Insulin content. The mean of all insulin contents measured was 45.4 ± 2.3 ng/islet (*n* = 56) and 49.5 ± 1.6 ng/islet (*n* = 54) for ventral and dorsal islets, respectively (not significantly different). Moreover, the insulin contents for ventral and dorsal islets were similar within the individual experiments (individual results not shown).

Glucagon content. The mean of all glucagon contents measured was 0.18 ± 0.02 ng/islet (*n* = 57) and 2.48 ± 0.19 ng/islet (*n* = 57) for ventral and dorsal islets, respectively (*P* < 0.001).

Total protein. The protein content of ventral islets, 0.67 ± 0.05 μg/islet (*n* = 35), was similar to that of dorsal islets, 0.72 ± 0.05 μg/islet (*n* = 34).

Cyclic AMP concentrations in ventral and dorsal islets

Islet cyclic AMP content and insulin secretion were measured after 2-min incubations. Dorsal islets contained more cyclic AMP than ventral islets at both 2.8 and 16.7 mM glucose (Fig. 1). The insulin secretion characteristics of dorsal and ventral islets were similar after 2-min incubations to those observed after 30-min incubations (see below), that is, at 2.8 mM glucose, insulin secretion was similar in dorsal and ventral islets, whereas dorsal islets secreted more insulin than ventral islets at 16.7 mM glucose (Fig. 1).

Effect of glucose on insulin secretion and proinsulin biosynthesis

Insulin secretion in the presence of 2.8 mM glucose in KRB-Hepes buffer was 2.3 ± 1.2 ng/10 islets per 30 min and 1.7 ± 0.5 ng/10 islets per 30 min for ventral and dorsal islets, respectively, and secretion was not stimulated above base line in the presence of 5.5 mM

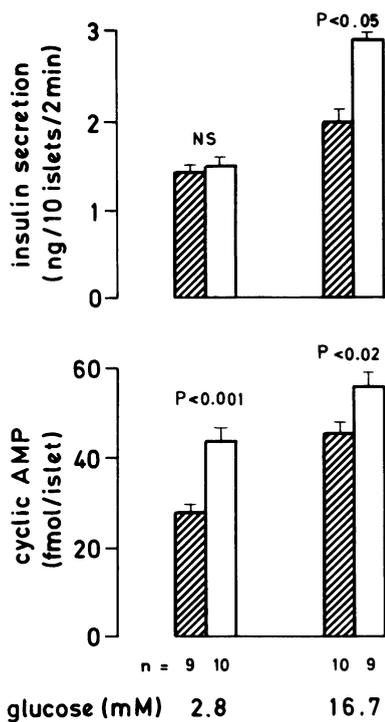


FIGURE 1 Cyclic AMP content and insulin release from islets isolated from the ventral, ▨ and dorsal, □ areas of the rat pancreas. Cyclic AMP content and insulin secretion were measured after short-term (2-min) incubations. Mean±SEM.

glucose (Fig. 2). When insulin secretion was stimulated above base line, i.e., at 16.7 mM glucose, ventral islets secreted significantly less insulin than dorsal islets (11.2 ± 1.1 ng/10 islets per 30 min and 32.2 ± 8.2 ng/10 islets per 30 min, respectively [$P < 0.025$, Fig. 2]).

Incorporation of [3 H]leucine into proinsulin was similar in the two islet types under basal conditions (i.e., 2.8 mM glucose, Fig. 2). At 5.5 mM glucose, proinsulin biosynthesis was stimulated above base line ($P < 0.02$ and $P < 0.001$ for ventral and dorsal islets, respectively). At this glucose concentration the [3 H]leucine incorporation into proinsulin was significantly greater in dorsal than in ventral islets. The rate of proinsulin biosynthesis was augmented further in the presence of 16.7 mM glucose and, again, was greater in dorsal than in ventral islets.

Because the rate of insulin secretion from dorsal islets was greater than that from ventral islets at 16.7 mM glucose, the effect of a higher glucose concentration was examined to determine whether further glucose-induced secretion could be obtained. It was found that insulin secretion from ventral and dorsal islets incubated at 30.0 mM glucose was $93.4 \pm 6.7\%$ ($n = 12$) and $94.4 \pm 3.4\%$ ($n = 12$), respectively, of that found

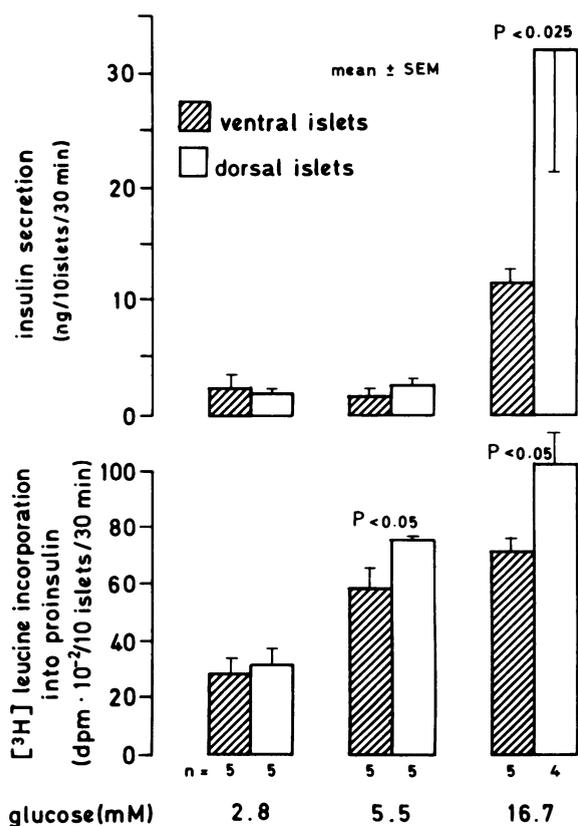


FIGURE 2 Glucose-induced insulin secretion and proinsulin biosynthesis by islets isolated from the ventral and dorsal areas of the rat pancreas. Proinsulin biosynthesis during 30-min incubations in the continuous presence of [3 H]leucine was measured as the tritium radioactivity in islet sonicates specifically bound by an excess of insulin antiserum.

in the presence of 16.7 mM glucose in the same experiments. At 30.0 mM glucose, the insulin secretion of dorsal islets remained significantly higher than that of ventral islets ($P < 0.01$). Proinsulin biosynthesis was not measured in these experiments.

Effect of exogenous glucagon on basal and glucose-stimulated insulin secretion and proinsulin biosynthesis

At 5.5 mM glucose the addition of 1 μ g/ml glucagon to the incubation buffer had no effect on insulin secretion from either ventral or dorsal islets (Fig. 3). As expected from the previous series of experiments (Fig. 2), in the presence of 16.7 mM glucose alone dorsal islets secreted more insulin than ventral islets. The addition of 1 μ g/ml glucagon to 16.7 mM glucose caused a twofold to threefold increase in insulin se-

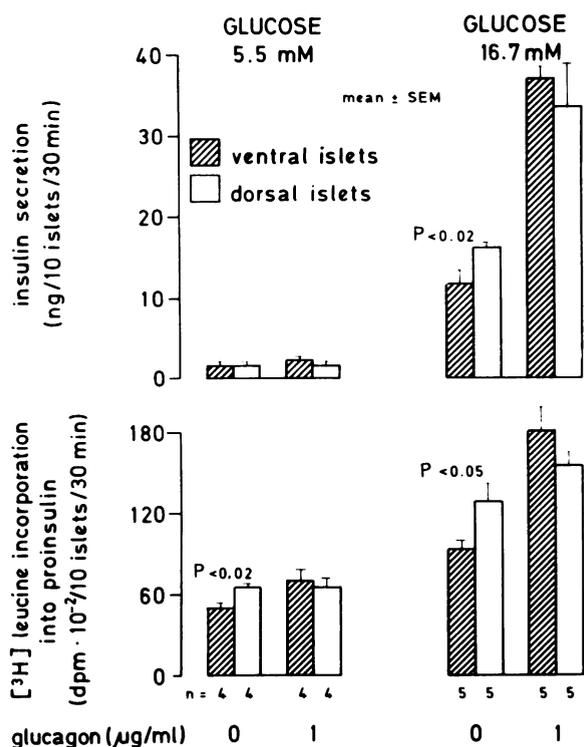


FIGURE 3 Effect of exogenous glucagon on insulin secretion and proinsulin biosynthesis by islets isolated from the ventral and dorsal areas of the rat pancreas. The effect of exogenous glucagon (1 $\mu\text{g}/\text{ml}$) on insulin secretion and proinsulin biosynthesis was measured after 30-min incubations in the presence of 5.5 mM or 16.7 mM glucose (separate experiments).

cretion from both types of islet, and under these conditions the previously noted differences in their insulin secretion were abolished (Fig. 3).

In the presence of 5.5 mM glucose, although the addition of 1 $\mu\text{g}/\text{ml}$ glucagon had no effect on dorsal islets, there was a tendency for increased [³H]leucine incorporation into proinsulin in ventral islets ($0.05 < P < 0.10$). The addition of glucagon (1 $\mu\text{g}/\text{ml}$) in the presence of 16.7 mM glucose increased the rate of proinsulin biosynthesis in ventral islets ($P < 0.005$); a similar tendency in dorsal islets did not achieve statistical significance. Thus, at 16.7 mM glucose, the addition of glucagon eliminated the difference in proinsulin biosynthesis (Fig. 2) between the two types of islet.

Effect of the phosphodiesterase inhibitor IBMX on glucose-stimulated insulin secretion

At a maximal phosphodiesterase-inhibiting concentration (1 mM), IBMX potentiated glucose-induced

insulin secretion from both ventral and dorsal islets (Table I). In the presence of 16.7 mM glucose and 1 mM IBMX, dorsal islets secreted more insulin than ventral islets ($P < 0.02$).

Processing of newly synthesized proinsulin

We have shown that over a 30-min period certain incubation conditions led to a greater proinsulin biosynthesis and a higher rate of insulin secretion in dorsal islets than in ventral islets. Because of these findings and because the main secretory product of the B cell is insulin rather than its precursor proinsulin, we investigated whether the rate at which newly synthesized proinsulin leaves the proinsulin pool is also greater in dorsal than in ventral islets. The results are shown in Table II. Because the rate of [³H]leucine incorporation into proinsulin was found to be greater in the presence of the amino acid-enriched DMEM than with KRB-Hepes (unpublished observations), the former medium was employed during the 15-min labeling period to maximize proinsulin biosynthesis during this period. After 15 min of labeling in DMEM with 16.7 mM glucose the specifically immunoprecipitable radioactivity in the islet sonicates in ventral and dorsal islets was not significantly different. (Note that this is a shorter labeling period than that used in previous experiments, in which after 30 min at 16.7 mM glucose there was a greater incorporation into proinsulin in the dorsal than in the ventral islets.) At the end of the 15-min labeling period column chromatography showed that all specifically immunoprecipitable radioactivity was in the proinsulin fraction. After a 30-min chase in KRB-Hepes buffer containing 2.8 mM glucose, 16.7 mM glucose, or 16.7 mM glucose plus 1 $\mu\text{g}/\text{ml}$ glucagon, ~60–70% of the specifically bound radioactivity remained in the proinsulin fraction (see Table II) of both ventral and dorsal islets. Under none of these conditions did the radioactivity remaining in newly synthesized proinsulin differ significantly between ventral and dorsal islets. Similarly, the amount of newly synthesized proinsulin remaining was not influenced by the various chase conditions.

ATP concentrations in ventral and dorsal islets

When glucose was omitted from the incubation buffer, the ATP concentration in ventral islets (4.4 ± 0.4 pmol/islet) was similar to that in dorsal islets (4.9 ± 0.4 pmol/islet). The ATP concentrations in both islet types increased with increasing glucose concentration (Fig. 4). In the presence of 2.8 and 16.7 mM glucose the

TABLE I
*Insulin Secretion in the Presence of 16.7 mM Glucose and the
 Phosphodiesterase Inhibitor IBMX*

Glucose	IBMX	Insulin secretion		
		Ventral islets	Dorsal islets	
		ng/10 islets · 30 min ⁻¹	ng/10 islets · 30 min ⁻¹	
16.7 mM	—	16.5±1.5 (n = 13)	P < 0.005	37.9±6.3 (n = 13)
16.7 mM	1 mM	48.4±6.1 (n = 13)	P < 0.02	73.2±7.8 (n = 13)

ATP concentrations in dorsal islets were greater than those in ventral islets.

Glucose utilization by ventral and dorsal islets

The rate of glucose utilization during 90-min incubations of islets was measured at 16.7 mM glucose by following the conversion of [5-³H]glucose into ³H₂O. It was found that glucose utilization by ventral islets was 605±75 pmol/10 islets per 90 min (n = 23), compared with that of the dorsal islets, which was 943±87 pmol/10 islets per 90 min (n = 22) (P < 0.05).

DISCUSSION

To compare B cell function in tissue of the ventral area of the rat pancreas with that of the dorsal area it was

TABLE II
Proinsulin Processing: [³H]Leucine in Proinsulin At the End
 of a 15-min Labeling Period and after a 30-min Chase*

	Ventral islets	Dorsal islets
	dpm · 10 ⁻² /10 islets	
After 15-min labeling †	75±6 (7)	91±7 (7)
After 30-min chase ‡		
2.8 mM glucose	44±3 (7)	50±3 (7)
16.7 mM glucose	43±5 (3)	55±10 (3)
16.7 mM glucose plus 1 µg/ml glucagon	53±2 (4)	52±6 (4)

The numbers in parentheses denote the number of observations.
 * [³H]Leucine in proinsulin was estimated by determining in islet sonicates the total radioactivity specifically bound by excess anti-insulin serum and then by measuring the proportions of radioactivity found in the proinsulin and insulin fractions, separated by column chromatography (see Methods).

† Labeling was carried out in DMEM containing no unlabeled leucine but supplemented with 100 µCi/ml [³H]leucine, 0.5 g/100 ml BSA and containing 16.7 mM glucose.

‡ During the chase period islets were incubated in KRB-Hepes buffer with the various test substances.

essential that a valid unit of reference be used. The islet appeared to be a useful reference unit, because size-matched islets from the two areas have similar insulin contents and a similar number of B cells (1). To compare the two islet types it was important to keep the ambient concentration of secreted hormones in the incubation medium relatively low, because the accumulation of extremely high concentrations of insulin (18) and glucagon (19) could modify the results. At present it is impossible to determine the ambient concentrations of insulin and glucagon in the extracellular fluid within the islets in vivo. Some recent work (20, 21) suggests that the concentrations of hormone measured in the mixed effluent of the pancreas may be diluted by blood from the exocrine pancreas by at least a factor of 10 between leaving the islet and reaching the portal vein. Because of these considerations, all experiments were carried out with relatively few islets in a relatively large volume of incubation medium (10 islets/ml). The similarities in the rate of insulin secretion by the two types of islet at low (2.8 mM) glucose and the differences in insulin secretion rate at high (16.7 mM) glucose have been observed under various experimental conditions: 2-min incubations, 30-min incubations, and in an islet perfusion system described previously (8). These considerations suggest that certain simple comparisons of the two islet types may be made by short-term-incubation experiments.

We (8) have shown that the greater glucagon content of dorsal islets is reflected in a greater glucagon secretion. Thus, during a 30-min incubation, each dorsal islet may secrete 30–40 pg of glucagon more than each ventral islet. It seems probable that the higher cyclic AMP content of dorsal islets found in the present experimental series is due to the greater glucagon content/secretion of such islets, which would presumably act through the adenylate cyclase-cyclic AMP system. The stimulant effects of glucagon on the B cell are glucose dependent (19, 22, 23), being more pronounced above the glucose threshold for stimulation of both insulin biosynthesis and secretion. Hence, al-

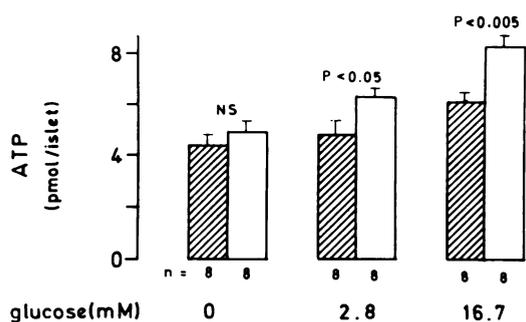


FIGURE 4 ATP content of pancreatic islets. Islets were preincubated for 30 min in KRB-Hepes containing 2.8 mM glucose. ATP content was measured at the end of 30-min incubations in KRB-Hepes containing 0, 2.8, or 16.7 mM glucose. Ventral islets, ▨; dorsal islets, □. Mean \pm SEM.

though cyclic AMP levels in dorsal islets were higher than in ventral islets, even at a low (2.8 mM) glucose concentration, the differences in the rates of proinsulin biosynthesis and insulin secretion between the two types of islet were seen only above the glucose threshold for stimulated biosynthesis and secretion. As expected (24), the glucose threshold for stimulation of proinsulin biosynthesis (below 5.5 mM glucose in these experiments) was lower than that for stimulation of insulin secretion. At 16.7 mM glucose the rates of both proinsulin biosynthesis and insulin secretion were greater in the glucagon-rich dorsal islet than in the ventral islet.

Insulin secretion at 30 mM glucose was similar to that at 16.7 mM for both ventral and dorsal islets, with dorsal islets secreting more insulin than ventral islets at both glucose concentrations. Thus, the maximum rate of insulin secretion was different in the two types of islet when glucose alone was used as the stimulus. The greater insulin secretion rate in the glucagon-rich dorsal islet may have been due to a greater activation of the adenylate cyclase-cyclic AMP system in this islet. Such an event would lead to potentiation of the glucose-stimulation effect. At stimulatory glucose concentrations the phosphodiesterase inhibitor IBMX was used to prevent cyclic AMP breakdown and hence to augment the effect of intrinsic activation of adenylate cyclase. By this means insulin release from both islet types was increased, and the secretion rate from dorsal islets remained greater than that from ventral islets. Experiments were designed to expose the B cells of the two types of islet to similar amounts of glucagon in the presence of 16.7 mM glucose. When a small amount of glucagon was added to the incubation medium of ventral islets, so that the total amount of glucagon that accumulated in the medium of the two types of islet was similar, insulin secretion by ventral

and dorsal islets was equalized (8). In the present experiments, when the adenylate cyclase system of B cells in both islet types was stimulated by a very high concentration of exogenous glucagon, so that the amount of glucagon secreted by either type of islet was insignificantly small compared with the amount added, the rate of both proinsulin biosynthesis and insulin secretion at 16.7 mM glucose were similar in the two islet types.

The results suggest that the observed differences in insulin secretion and biosynthesis by ventral and dorsal islets during glucose stimulation may indeed be due to differences in activation of adenylate cyclase by factors extrinsic to the B cell rather than to intrinsic B cell differences. Islet components other than glucagon, such as vasoactive intestinal peptide and β -adrenergic agonists activate the adenylate cyclase system, but the relative distributions of these substances in the two islet types, and hence their relative contributions to the cyclic AMP concentration, are unknown. Although somatostatin can inhibit cyclic AMP accumulation in the islet (25), it is secreted in similar quantities by the two types of islet, at both low and high glucose concentrations (8). It has been reported that pancreatic polypeptide does not affect tissue cyclic AMP levels (26). (In addition, the effect of pancreatic polypeptide on insulin release has not been firmly established; both no effect [27] and an inhibitory effect [28] of this hormone having been reported.)

When islets from the whole pancreas were considered together (14), the rate of glucose metabolism by the islets was found to be directly proportional to the rate of glucose-stimulated insulin release. In the present experimental series, in which glucose utilization rates in the islet were measured at stimulatory glucose concentrations, the utilization rate of the dorsal islet was elevated above that of the ventral islet in proportion to the greater insulin secretion of the dorsal islet. Thus, the efficiency with which glucose utilization is coupled to insulin secretion appears to be similar in the two islet types. In turn, the higher ATP content of dorsal islets may be, at least in part, a reflection of a higher glycolytic flux in these islets.

Although the rates of insulin biosynthesis and secretion were higher in dorsal than in ventral islets under certain conditions, there was no apparent difference in the rates at which newly synthesized proinsulin left the proinsulin pool through a combination of conversion to insulin and direct secretion. However, the design of the experiments did not allow the rate of processing of previously synthesized, unlabeled proinsulin to be measured. Others who have used immunoprecipitation methods to measure the rate of proinsulin conversion to insulin found, as reported here, that

the extracellular glucose concentration had no apparent effect on this process (29).

In summary, the results are consistent with the possibility that, within the islet, glucagon has a potentiating effect on glucose-stimulated insulin biosynthesis and secretion. Whether such glucagon effects are normally mediated exclusively via a higher ambient concentration of the hormone in the extracellular space of the dorsal islet, as probed in the present study, is not clear. An additional, or alternative, possibility is that information may be transmitted from one cell to another by direct intercellular communication (4, 6, 7). Finally, the experiments carried out to date have not revealed intrinsic differences between the B cells of the two islet types. The question remains as to the physiological implications of two functionally distinct subpopulations of islets. Blood leaving one type of islet enters a portal system serving the adjacent exocrine pancreas before joining the portal vein, where it mixes with blood from the other pancreatic area (21, 30). Thus, it is at the level of the exocrine pancreas that the functional differences between the two islet types are most probably expressed, and, indeed, all four major pancreatic hormones do affect exocrine function (20, 31–34). However, differences in exocrine function between the dorsal and ventral pancreas have yet to be demonstrated.

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REFERENCES

- Baetens, D., F. Malaisse-Lagae, A. Perrelet, and L. Orci. 1979. Endocrine pancreas: three-dimensional reconstruction shows two types of islets of Langerhans. *Science (Wash. D. C.)* **206**: 1323–1325.
- Orci, L., and R. H. Unger. 1975. Hypothesis: functional subdivisions of the islets of Langerhans and the possible role of D-cells. *Lancet*. **II**: 1243–1244.
- Unger, R. H., R. E. Dobbs, and L. Orci. 1978. Insulin, glucagon and somatostatin secretion. *Annu. Rev. Physiol.* **40**: 307–343.
- Orci, L., F. Malaisse-Lagae, M. Ravazzola, D. Rouiller, A. E. Renold, A. Perrelet, and R. Unger. 1975. A morphological basis for intercellular communication between α - and β -cells in the endocrine pancreas. *J. Clin. Invest.* **56**: 1066–1070.
- Meissner, H. P. 1976. Electrophysiological evidence for coupling between beta cells of pancreatic islets. *Nature (Lond.)* **262**: 502–504.
- Kohen, E., C. Kohen, B. Thorell, D. H. Mintz, and A. Rabinovitch. 1979. Intercellular communication in pancreatic islet monolayer cultures: a microfluorometric study. *Science (Wash. D. C.)* **204**: 862–865.
- Meda, P., J.-F. Deneff, A. Perrelet, and L. Orci. 1980. Nonrandom distribution of gap junctions between pancreatic β -cells. *Am. J. Physiol.* **238**: C114–C119.
- Trimble, E. R., and A. E. Renold. 1981. Ventral and dorsal areas of the rat pancreas: islet hormone content and secretion. *Am. J. Physiol.* **240**: E422–E427.
- Lacy, P. E., and M. Kostianovsky. 1967. Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes*. **16**: 35–39.
- Halban, P. A., C. B. Wollheim, B. Blondel, and A. E. Renold. 1980. Long-term exposure of isolated pancreatic islets to mannoheptulose: evidence for insulin degradation in the β cell. *Biochem. Pharmacol.* **29**: 2625–2633.
- Cailla, H. L., M. S. Racine-Weisbuch, and M. A. De-laage. 1973. Adenosine 3',5'cyclic monophosphate assay at 10^{-15} mole level. *Anal. Biochem.* **26**: 394–407.
- Stanley, P. E., and S. G. Williams. 1969. Use of the liquid scintillation spectrometer for determining adenosine triphosphate by the luciferase enzyme. *Anal. Biochem.* **29**: 381–392.
- Verspohl, E. J., M. Händel, and H. P. T. Ammon. 1979. Pentose-phosphate shunt activity of rat pancreatic islets: its dependence on glucose concentration. *Endocrinology*. **105**: 1269–1274.
- Zawalich, W. S., and F. M. Matschinsky. 1977. Sequential analysis of the releasing and fuel function of glucose in isolated perfused pancreatic islets. *Endocrinology*. **100**: 1–8.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275.
- Herbert, V., K.-S. Lau, C. W. Gottlieb, and S. J. Bleicher. 1965. Coated charcoal immunoassay of insulin. *J. Clin. Endocrinol. Metab.* **25**: 1375–1384.
- Tager, H. S. 1976. Coupling of peptides to albumin with difluorodinitrobenzene. *Anal. Biochem.* **71**: 367–375.
- Ammon, H. P. T., and E. Verspohl. 1976. Pyridine nucleotide in pancreatic islets during inhibition of insulin release by exogenous insulin. *Endocrinology*. **99**: 1469–1476.
- Schauder, P., J. Arends, B. Schindler, R. Ebert, and H. Frerichs. 1977. Permissive effect of glucose on the glucagon-induced accumulation of cAMP in isolated rat pancreatic islets. *Diabetologia*. **13**: 171–175.
- Saito, A., J. A. Williams, and T. Kanno. 1980. Potentiation of cholecystokinin-induced exocrine secretion by both exogenous and endogenous insulin in isolated and perfused rat pancreata. *J. Clin. Invest.* **65**: 777–782.
- Lifson, N., K. G. Kramlinger, R. R. Mayrand, and E. J. Lender. 1980. Blood flow to the rabbit pancreas with special reference to the islets of Langerhans. *Gastroenterology*. **79**: 466–473.
- Katada, T., and M. Ui. 1979. Islet-activating protein: enhanced insulin secretion and cyclic AMP accumulation in pancreatic islets due to activation of native calcium ionophores. *J. Biol. Chem.* **254**: 469–479.
- Wollheim, C. B., E. G. Siegel, M. Kikuchi, A. E. Renold, and G. W. C. Sharp. 1980. The role of extracellular Ca^{++} and islet calcium stores in the regulation of biphasic insulin release. In *Biochemistry and Biophysics of the Pancreatic β -Cell*. W. J. Malaisse and I.-B. Täljedal, editors. *Horm. Metab. Res. Suppl.* **10**: 108–115.
- Ashcroft, S. J. H., J. Bunce, M. Lowry, S. E. Hansen, and C. J. Hedekov. 1978. The effect of sugars on (pro)insulin biosynthesis. *Biochem. J.* **174**: 517–526.

25. Claro, A., V. Grill, S. Efendic, and R. Luft. 1977. Studies on the mechanisms of somatostatin action on insulin release. *Acta Endocrinol.* **85**: 379-388.
26. Schwartz, S. S., B. Corkey, J. R. Williamson, and A. H. Rubenstein. 1980. Effect of bovine pancreatic polypeptide on isolated rat liver cells. *Endocrinology.* **106**: 1178-1181.
27. Adrian, T. E., S. R. Bloom, K. Hermansen, and J. Iversen. 1978. Pancreatic polypeptide, glucagon and insulin secretion from the isolated perfused canine pancreas. *Diabetologia.* **14**: 413-417.
28. Lundquist, I., F. Sundler, B. Ahrén, J. Alumets, and R. Håkanson. 1979. Somatostatin, pancreatic polypeptide, substance P and neurotensin: cellular distribution and effects on stimulated insulin secretion in the mouse. *Endocrinology.* **104**: 832-838.
29. Pipeleers, D. G., M. Marichal, and W. J. Malaisse. 1973. The stimulus-secretion coupling of glucose-induced insulin release. XIV. Glucose regulation of insular biosynthetic activity. *Endocrinology.* **93**: 1001-1011.
30. Fraser, P. A., and J. R. Henderson. 1980. The arrangement of endocrine and exocrine pancreatic microcirculation observed in the living rabbit. *Q. J. Exp. Physiol.* **65**: 151-158.
31. Söling, H. D., and K. O. Unger. 1972. The role of insulin in the regulation of α -amylase synthesis in the rat pancreas. *Eur. J. Clin. Invest.* **2**: 199-212.
32. Dyck, W. P., E. C. Texter, J. M. Lasater, and N. C. Hightower. 1970. Influence of glucagon on pancreatic exocrine secretion in man. *Gastroenterology.* **58**: 532-539.
33. Raptis, S., W. Schlegel, E. Lehmann, H. C. Dollinger, and C. Zoupas. 1978. Effects of somatostatin on the exocrine pancreas and the release of duodenal hormones. *Metab. Clin. Exp.* **27**: 1321-1328.
34. Lin, T-M., D. C. Evans, R. E. Chance, and G. F. Spray. 1977. Bovine pancreatic peptide: action on gastric and pancreatic secretion in dogs. *Am. J. Physiol.* **232**: E311-E315.