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Reversible Inhibition by Hyaluronidase of the Insulinotropic Action of Tolbutamide in Isolated Hamster Pancreatic Islets[1] (37839)

I. A. MACCHI, FÜSÛN N. ZEYTINOĞLU, AND SAMUEL B. BEASER
Department of Biology, Boston University, Boston, Massachusetts 02215

During the course of investigating the mechanism whereby the sulfonylureas stimulate insulin release, we have observed that hamster pancreatic islets isolated by means of collagenase in combination with hyaluronidase are insenstitive to the insulinotropic action of tolbutamide under conditions wherein the rate of immunoreactive insulin (IRI) release was increased significantly in the presence of a maximally effective concentration of glucose. The present report presents evidence demonstrating such selective inhibition and its subsequent reversal.

Materials and Methods. Islet isolation and incubation. Islets were isolated from pancreatic tissue of the female golden hamster (84–109 g body wt) basically as described by Lacy and Kostianovsky (1) employing 3 ml/pancreas of Hanks balanced salt solution (2) containing collagenase (Worthington, Lot CLS-901, 125–168 U/mg) at 2 mg/ml plus hyaluronidase (1.69 μg/ml; Sigma, Lot 18B-0650) or at 4 mg/ml but excluding hyaluronidase.

Islets were incubated using a system similar to that described by Keen, Field, and Pastan (3). A Teflon receptacle equipped with a 400-mesh nylon screen (Pharmacia) bottom and fitting within the center well of this system was used to facilitate transfer of islets to fresh medium during the course of incubation. For each determination, five islets were placed within the receptacle in the center well and incubated at 37° and 72 cycles/min in a 95% O_2–5% CO_2 gas phase in 0.5 ml Krebs–Ringer–bicarbonate medium (pH 7.4) containing Armour bovine serum albumin, fraction V (2 mg/ml). Islets were preincubated in the presence of glucose (0.9 mg/ml) for 30 min or, as otherwise indicated, for 6 hr with renewal of the medium at hourly intervals. Incubation was continued for 15 min or 1 hr in fresh medium. In one series, the medium contained graded concentrations of glucose (0.9–2.5 mg/ml) with or without inclusion of sodium tolbutamide[2] (1 mg/ml). Otherwise, the medium contained glucose (0.9 or 3.0 mg/ml) or sodium tolbutamide (1 mg/ml) plus glucose (0.9 mg/ml). The presence of five islets within each receptacle was verified microscopically at the beginning and at the end of the incubation period. In each series, each experiment was performed in duplicate employing islets derived from a common tissue pool.

Immunoassay and statistical analysis. Immunoreactive insulin was measured as described previously (4) using bovine insulin (Lilly, Lot PJ 4609) as a standard and suitable aliquots of medium from individual incubation vessels. Each assay was performed in duplicate, and the IRI values expressed as indicated below.

Data were analyzed by simple analysis of variance and Dunnett's test (5), and the levels of significance at the 0.5 or 0.01 confidence levels were derived from Dunnett's tables (6). Otherwise, as indicated, the significance of differences between means was computed by "Student's" t distribution for small-sample analysis of nonpaired experiments (7).

Results. Exposure of hamster pancreatic islets to hyaluronidase during isolation sup-

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[2] Orinase Diagnostic, Lot PA592 JG, The Upjohn Co., Kalamazoo, MI.

TABLE I. Effect of Tolbutamide on IRI Release Rate of Hamster Pancreatic Islets Isolated by Means of Collagenase in the Absence or Presence of Hyaluronidase.

Enzymatic isolation procedure	IRI (μU/5 islets/hr)[a] Glucose (mg/ml) 0.9	Glucose (mg/ml) 3.0	Tolbutamide (1 mg/ml)
Collagenase	2019 ± 345	4363 ± 932[b]	3369 ± 1045[c]
Collagenase + hyaluronidase	1563 ± 310	2756 ± 603[b]	1456 ± 279[d]

[a] Each value is the mean ± SE for 8 determinations representing 4 experiments.
[b,c,d] Significance of difference compared with respective value for glucose at 0.9 mg/ml: [b] $P < .01$; [c] $0.01 < P < 0.05$; [d] $P > 0.05$.

presses the insulinotropic action of tolbutamide but not that of elevated glucose concentration during subsequent incubation. Table I demonstrates that the average IRI release rate in the presence of tolbutamide was not significantly different from the corresponding value for glucose at 0.9 mg/ml in islets isolated by means of collagenase in combination with hyaluronidase in contrast to those not exposed to hyaluronidase. These data show, moreover, that the average IRI release rate was significantly greater in the presence of glucose at 3.0 mg/ml compared to the corresponding value for glucose at 0.9 mg/ml, irrespective of previous exposure of islets to hyaluronidase.

Failure of tolbutamide to increase significantly the rate of IRI release by isolated hamster pancreatic islets previously exposed to hyaluronidase does not appear to be a function of the concentration of glucose used adjunctly during incubation (Table II). These data show that the average IRI release rate is not increased significantly by tolbutamide in the presence of graded concentrations of glucose relative to corresponding IRI values in the absence of tolbutamide. They illustrate also that the IRI release rate of islets exposed to hyaluronidase increases progressively with increasing glucose concentration and peaks in the presence of glucose at 2.0 mg/ml.

Suppression of the insulinotropic action of tolbutamide in isolated hamster pancreatic islets previously exposed to hyaluronidase was reversed by prolonged incubation of the islets in Krebs–Ringer medium (Table III). These data show that the average IRI release rate in the presence of tolbutamide was significantly greater than the corresponding value in the presence of glucose at 0.9 mg/ml following preincubation for 6 hr but not following preincubation for 30 min. They demonstrate, moreover, that under both conditions of preincubation, the average IRI release rate was increased significantly in the presence of glucose at 3.0 mg/ml relative to corresponding values for glucose at 0.9 mg/ml.

Discussion. Present findings indicate that exposure of hamster pancreatic islets to a low concentration of hyaluronidase during isolation by means of collagenase causes reversible inhibition of the insulinotropic action of tolbutamide without affecting glucose-stimulated insulin secretion. This inhibition is not attributable to the collagenase since the IRI release rate of islets isolated by this enzymatic procedure in the absence of

TABLE II. Effect of Graded Concentrations of Glucose With or Without Inclusion of Tolbutamide on IRI Release Rate of Hamster Pancreatic Islets Exposed to Hyaluronidase.

Glucose (mg/ml)	IRI (μU/5 islets/hr)[a] Without tolbutamide	Tolbutamide (1 mg/ml)	Change	P[b]
0.9	1642 ± 277	—	—	—
1.5	3633 ± 812*	3000 ± 291	−633	>0.05
2.0	4417 ± 585*	3029 ± 595	−1388	>0.05
2.5	3633 ± 379*	3267 ± 712	−366	>0.05

[a] Each value is the mean ± SE obtained in 6 determinations representing 3 experiments.
[b] Statistical significance analyzed by "Student's" t test.
* Significance of difference compared with value for glucose at 0.9 mg/ml, $P < 0.01$.

TABLE III. Reversal of the Inhibition by Hyaluronidase of the Insulinotropic Action of Tolbutamide in Isolated Hamster Pancreatic Islets.

Tolbutamide (mg/ml)	Glucose (mg/ml)	(IRI μU/5 islets/15 min)[a] Preincubated 30 min	Preincubated 6 hr
None	0.9	593 ± 72	103 ± 17
None	3.0	1025 ± 360[b]	192 ± 28[b]
1	0.9	362 ± 34[c]	256 ± 30[d]

[a] Each value is the mean ± SE obtained in 8 determinations representing 4 experiments.

[b,c,d] Significance of difference compared with respective value for glucose at 0.9 mg/ml: [b]$0.01 < P < 0.05$; [c]$P > 0.05$; [d]$P < 0.01$.

hyaluronidase was increased significantly in the presence of tolbutamide. It appears also that the IRI release rate *in vitro* of islets exposed to hyaluronidase is a function of glucose concentration essentially as reported for incubated isolated rat pancreatic islets (8, 9) and pieces of Chinese hamster pancreas (10). Work in progress in our laboratory indicates tentatively that hamster pancreatic islets isolated in the presence of hyaluronidase are insensitive also to the insulinotropic action *in vitro* of several sulfonylureas chemically different from tolbutamide. We have not determined yet, however, whether this is a species-specific phenomenon in view of findings suggesting the existence of species differences in the responsiveness of isolated pancreatic islets to the insulinotropic action of tolbutamide (11). We also have not yet investigated whether the suppressive effect of hyaluronidase is demonstrable with insulinotropic agents other than the sulfonylureas.

The inhibitory action of hyaluronidase on the insulinotropic action of tolbutamide may be exerted through a mechanism involving a cellular receptor for tolbutamide, possibly associated with the beta cell plasma membrane, different from that for glucose. This interpretation is consistent with reported evidence construed as suggesting the existence of a beta cell plasma membrane receptor for tolbutamide in microdissected pancreatic islets of the obese hyperglycemic mouse (12, 13). Moreover, the unmasking by proteolysis of a beta cell membrane receptor for glucose is suggested by the recent demonstration that mild pronase treatment of isolated rat pancreatic islets reversibly increases glucose-stimulated IRI release *in vitro* (14). Assuming the existence of a beta cell plasma membrane receptor for tolbutamide, hyaluronidase may exert its effect by impeding transport of tolbutamide to the receptor or, most likely, by a reversible modification of the molecular structure of the receptor. These proposed modes of action would be consistent with the suggestion that hyaluronidase of mammalian origin alters the integrity of cell membranes by depolymerizing the long-chain mucopolysaccharides of the intercellular ground substance (15) and by modifying the tertiary structure of membrane polypeptides (16). Whichever of the proposed modes of action is considered, activation of the receptor through interaction with the tolbutamide molecule conceivably would be precluded and thus might prevent calcium uptake which has been linked with tolbutamide-stimulated insulin release (17).

Summary. Exposure of hamster pancreatic islets to a low concentration of hyaluronidase during isolation by means of collagenase causes reversible inhibition of the insulinotropic action of tolbutamide without apparently affecting glucose-stimulated insulin secretion. This inhibition is not attributable to the collagenase. These findings suggest that the inhibitory action of hyaluronidase on the insulinotropic action of tolbutamide may be exerted through a mechanism involving a cellular receptor for tolbutamide, possibly associated with the beta cell plasma membrane, different from that for glucose.

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