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Lessons From In Vitro Perifusion of Pancreatic Islets Isolated From 80 Human Pancreases

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We report the average insulin response to acute glucose measured by in vitro perifusion of pancreatic islets isolated from 80 consecutive human organs. Different perifusion parameters were considered [basal release, stimulation index (SI), time to peak, incremental area under the curve Δ-AUC*a*)], and the correlation among them was determined. SI positively correlated with Δ-AUC*a* ($p < 0.001$, $r = 0.80$) while negatively with time to peak ($p < 0.05$, $r = -0.23$). We also evaluated several variables of the isolation procedure that might affect responsiveness to glucose by human islets. Sex and age of pancreas donors, cold ischemia time, duration of the digestion, collagenase concentration, and lot characteristics (collagenase, trypsin, clostripain, and proteases activity), and final islet yield were considered. Multivariate regression analysis showed only an independent association between SI and the concentration of collagenase ($p = 0.01$).

Key words: Perifusion parameters; Insulin response; Pancreatic islets

INTRODUCTION

In vitro perifusion is a widely utilized method to study secretagogue-induced insulin release by isolated pancreatic islets. In contrast to static incubation, perifusion permits evaluation of the dynamics of insulin release in a situation where feedback inhibitory/stimulatory signals of released hormone(s) are avoided (6). Various parameters have been used to quantitatively and qualitatively express the secretion of insulin during perifusion, among them: perifusion index (1), Δ area under the curve (2), and stimulation index (SI) (3,4). However, it is still uncertain which of them better describes islet responsiveness to a given stimulus (6). Moreover, with the same term (i.e., SI) two different concepts are sometimes intended (3,4). Lack of standardization of perifusion parameters and results makes it difficult to compare data obtained in different laboratories. Thus, the identification of standardized protocols for the interpretation of the data could be helpful, especially when perifusions are performed as control quality test of a human islet preparation (6). In this study we identified individual parameters of a perifusion experiment and analyzed the correlation between them. The same parameters were utilized to determine which of the variables of the isolation procedure mainly affect the function in vitro of isolated human islets.

MATERIALS AND METHODS

Islets were isolated from pancreases obtained from heart-beating cadaveric multiorgan donors using a modification of an automated method (7). Data were obtained from 80 consecutive preparations (with a purity ≥50%). Enzymatic digestion of the pancreases was obtained by using type P collagenase (Boheringer Mannheim, Germany) of nine different lots with different collagenase activities and differences in the contaminant enzymes such as clostripan, trypsin, and protease activity (Table 1).

After isolation, islets were cultured at 37°C in 5% CO_2 in M199 supplemented with 10% fetal calf serum, 1% antibiotics, and 1% glutamine (2). After an overnight culture, 200 hand-picked islets were perifused at 37°C with an oxygenated (95% O_2/5% CO_2) Krebs-Ringer bicarbonate buffer plus 0.5% BSA (Sigma Chemical Co., St. Louis, MO). After 40 min at 3.3 mM glucose (equilibrating period) islets were acutely stimulated for 20 min with 16.7 mM glucose (stimulating period). Each experiment was performed at least in duplicate. Insulin secretion in the perfusate was assayed by radioimmunoassay (Insulin RIA Kits, Incstar, Stillwater, MN) as previously described (2).

The perifusion secretory parameters considered were the following: basal release; maximal incremental in-

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Table 1. Characteristics of the Different Lots of Collagenase Utilized

Lot of Collagenase*	Collagenase Activity (U/mg lyo)	Clostripain Activity (U/mg lyo)	Proteases Activity (U/mg)	Trypsin Activity (U/mg lyo)
P121458 (13)	2.7	38.7	152	1.55
P129126 (14)	1.65	31.6	72.5	1.18
P130494 (9)	3	18.4	138	0.77
P134938 (10)	2.61	8.1	128	1.1
P138592 (6)	2.67	2.97	33.7	0.26
P140396 (7)	2.14	4.9	55.7	0.37
P140398 (10)	2.3	7.94	80.8	1.08
P146278 (6)	1.61	3.1	63.9	0.07
P146279 (5)	1.55	2.1	50.5	0.07

*Collagenase lot codes, with the number of isolations performed with each lot in parentheses.

crease from basal (SI = peak/basal release) (1,4); time to peak (minutes necessary to reach the maximal stimulation after the shift to high glucose); absolute incremental area under the curve after 20 min of stimulation [Δ-AUC*a*, expressed as pg/islet/20 min] (2); and relative incremental area under the curve (expressed in terms of percentual change from basal, Δ-AUC*r*) (9).

The following isolation variables were taken into account: sex and age of pancreas donors; cold ischemia time of the pancreases; duration of the digestion; collagenase concentration and lot characteristics; and final islet yield expressed in absolute and in equivalent terms.

Possible correlations between the parameters utilized to quantitatively and qualitatively assess the release of insulin were assessed by Spearman's correlation. The effect of isolation variables on insulin release was analyzed by a univariate analysis. Finally, a multivariate regression analysis including all the considered variables was performed to confirm independent associations.

RESULTS AND DISCUSSION

Insulin release in response to acute glucose by human islets isolated in 80 consecutive isolations was biphasic (Fig. 1). Basal release (mean 5.1, median 4.2, range 0.3–20.2 pg/islet/min) remained stable for the last 20 min of the equilibration period. Insulin responsiveness to glucose was expressed by the following parameters: stimulation index (mean 2.8, median 2.2, range 0.7–32.3), time to peak (mean 6.1, median 4.6, range 1–15 min), Δ-AUC*r* (mean 32.3, median 10.3, range 8.2–384.8), and Δ-AUC*a* (mean 336, median 301, range 10–1158 pg/islet/20 min). SI, which depicts the first phase of insulin release, was positively correlated to Δ-AUC*a*, which principally represents the second phase ($p < 0.001$, $r = 0.80$). Conversely, SI was negatively correlated with time to peak ($p < 0.05$, $r = -0.23$). Δ-AUC*r* highly correlated with Δ-AUC*a* ($p < 0.001$, $r = 0.81$).

Isolation variables were the following: donor age (mean 35, median 37, range 13–64 years old, 36 male and 44 female), cold ischemia time (mean 4.7, median 4.0, range 0–12 h), absolute islet yield (mean 148,000, median 190,000, range 10,000–848,000) and equivalent islet yield (mean 173,000, median 243,000, range 10,000–625,000), and collagenase concentration (mean 2.8, median 3.0, range 1–5 mg/dl). After proper logaritmic transformation, a significant association (univariate analyses) was observed between SI (Δ-AUC*r*) and collagenase concentration, and islet yield. Also trypsin and clostripain activities were inversely correlated to the islet yield ($p = 0.01$). However, multivariate regression analysis confirmed the independent association only between SI (Δ-AUC*r*) and collagenase concentration ($p = 0.01$).

Dynamics of insulin release were biphasic with the magnitude of the first (SI) and second phase (Δ-AUC*r*) significantly correlated together, and with the precocity of the peak (time to peak). Thus, whenever a good SI was achieved, it fell within the first 5 min after the stimulus, and was followed by a valid second phase. In addition, SI was highly correlated with all the other secretory parameters, indicating that it can be used as the only parameter to determine islet responsiveness. Δ-AUC*r* was highly correlated with Δ-AUC*a*, demonstrating that it is possible to reliably estimate the secretory activity without knowing the precise number of perifused islets. Finally, the first 20 min of the equilibration period were enough to achieve a steady state of insulin release, thus indicating that the length of perifusions performed as quality control tests can be reduced overall to 30 min (Fig. 1), leading to considerable savings in time and money.

Islet responsiveness to glucose inversely correlated with collagenase concentration, confirming that enzymatic digestion is the critical step in the isolation procedure (6). The negative correlation between collagenase

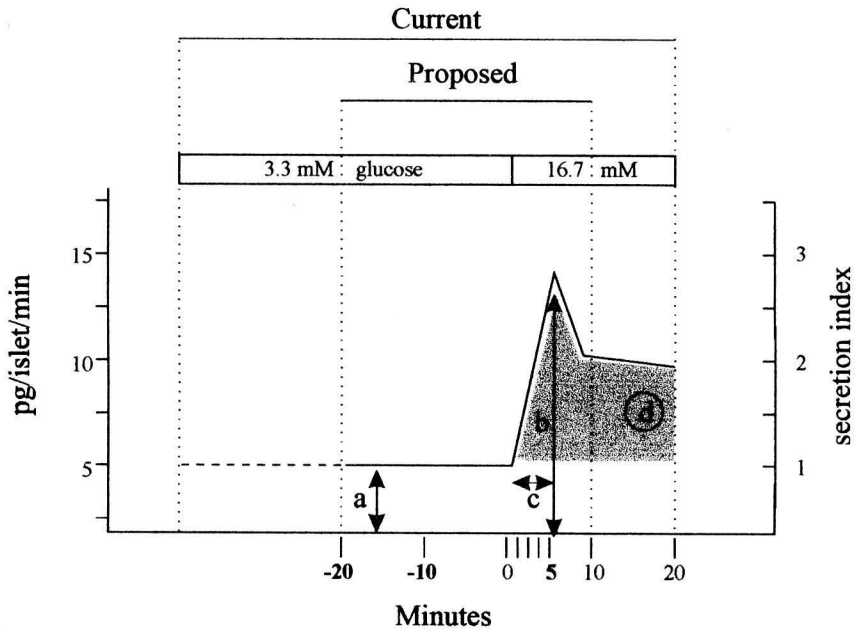

Figure 1. Mean values of insulin release by 80 consecutive human islet preparations submitted to an acute glucose challenge by in vitro perifusion. Note that insulin release remained stable in the last 20 min of equilibration period averaging 5.1 pg/islet/min (a) at time 0; 6.1 min after the shift to high glucose (c), insulin release reached the peak of 14.3 pg/islet/min (SI of 2.8, b). Insulin secretion, expressed as absolute incremental area under the curve (Δ-AUC*a*, in gray area, d) was of 336 pg/islet/20 min, and correlated positively with the Δ-AUC expressed in terms of percentual change from basal (Δ-AUC*r*) of 32.3 arbitrary units. Values are means.

concentration and islet function was independent from trypsin or proteases activities, in contrast to what has been previously reported in rodent islets (10). At this regard, however, it should be pointed out that trypsin activity was remarkably low in all the collagenase lots utilized by us. The activity of contaminant proteases did not affected human islet yield, but other collagenase contaminants (i.e., endotoxins) might affect islet viability and function (8). Lack of association between donor organ characteristics and islet function contrasts with the previous finding that islets isolated from older donors have impaired responsiveness to glucose (5). Glucose-induced insulin release was also independent from cold ischemia time, which, in our series of isolations, was limited to a maximum of 12 h. Other reports showed blunted responses from islets isolated from pancreas with more prolonged periods of cold storage (4). Finally, univariate analysis showed an inverse relationship between SI and islet yield, raising the question as to whether it is preferable to pursue the highest islet yield even at the cost of a reduced beta cell function.

Although the final decision regarding the quality of a given islet preparation must relay on cell viability assays, insulin over total DNA or protein content, and histology (6), this study reinforces the notion that perifusion represents an additional informative quality test; in this regard, a short practical perifusion protocol is recommended.

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