Addendum

Rat islet isolation yield and function are donor strain dependent

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Laboratory animals, in press

Summary

Effective rat islet isolation is pertinent for successful islet transplantation and islet studies *in vitro*. To determine which rat strain yields the highest number of pure and functional islets, four commonly used rat trains were compared with regard to islet yield, islet purity and islet function. Secretory responses were assessed by stimulation with glucose, and by stimulation with glucose plus 3-isobutyl-1-methylxanthine (IBMX). We show that rat islet function and isolation yield are donor strain dependent. Albino Oxford (AO) rats donated twice as many islets than Wistar, Lewis and Sprague Dawley (SD) rats. Stimulation with glucose plus IBMX resulted in an average five-fold increase of the stimulation index of AO, Lewis, Wistar and SD rats compared to stimulation with glucose only. AO islets had improved secretory responses after a one-week culture period, but required addition of IBMX to glucose to elicit a distinguished stimulated insulin secretion after two days of culture. Islets from SD rats showed inferior results with regard to purity immediately after isolation and with regard to function after short and after long time culture. Because Lewis islets possessed the highest secretory response to glucose (without IBMX) immediately after isolation, Lewis rats may be preferred as islet donor. Addition of IBMX to glucose for *in vitro* functional testing is recommended because it warrants high insulin secretory responses of islets regardless what rat strain. AO rats are then preferred as islet donor since the number of experimental animals is reduced twofold compared to Lewis, Wistar and SD rats.

Introduction

Islet transplantation has proven to be a potential cure for IDDM patients (10). Islet isolation is crucial for successful islet transplantation. Islet mass, islet quality and islet purity are determined by islet isolation efficacy, which depends on many factors, among which organ procurement, collagenase digestion and purification method (5, 18, 21). Apart from differences in islet yield among species (16, 19), also variability in islet yield within one species is found (13). These variations can be attributed to organ quality (15), batch-to-batch variation in collagenase preparation (22, 23), donor age (6), but could also be strain-dependent (1). Over the past years islet isolations in our laboratory were performed with different rat strains. Islet isolation yields of Albino Oxford rats were almost twice as high compared to Wistar rats. To determine which rat strain in yields the highest number of pure and functional islets, four commonly used rat strains were compared in the present study with regard to islet yield and islet function. Considering efficient animal use, the rat strain with the highest islet isolation yield may well lead to a reduction of the number of laboratory animals for islet experiments.

Cultured islets exhibit a poor secretory response to glucose. Therefore, glucose stimulated insulin secretion is often tested with glucose in combination with chemicals like 3-isobutyl-1-methylxanthine (IBMX) and theophylline (2, 3, 7, 8). IBMX is a phosphodiesterase inhibitor that elevates the intracellular cAMP, which increases the release of free Ca^{2+} and consequently the release of insulin (4, 11, 12). To determine the necessity of IBMX for the assessment of islet function *in vitro*, glucose challenge tests in the present study were performed with and without IBMX.

Materials and methods

Design of the study

Albino Oxford (AO), Lewis, Wistar and Sprague Dawley (SD) rats served as islet source. Islets were isolated with a collagenase and purified by a dextran gradient. The number of islets per isolation was assessed during handpicking. Islet function was determined by a static glucose challenge test immediately after isolation, after a short period of culture (two days), and after a long period of culture (seven days). The glucose challenge test was performed both in the absence and in the presence of IBMX.

Animals

Male inbred Albino Oxford rats, inbred Lewis rats, outbred Wistar rats,

and outbred Sprague Dawley rats were purchased from Harlan (Harlan CPB, Zeist, The Netherlands). Animals were specific pathogen free and housed in groups of 2-3 animals per cage (macrolon). All rats were housed for at least one week (12 hrs light/12 hrs dark cycle) and served as islet source once they reached the weight between 290 and 310 gram. Food (standard 10 mm rodent chow, RMH-B, Hopefarms, Woerden, the Netherlands) and water (pH 4.5, acidified with HCl) were available *ad libitum*. All animals received care in compliance with the guidelines of the local Animal Care and Use Committee following National Institutes of Health guidelines. Rats were killed under halothane anesthesia (with O_2 and N_2O in a 2:1 ratio) after excision of the pancreas.

Islet isolation

Islets were isolated as previously described (23). Briefly, under halothane anesthesia, the abdomen was opened and the common bile duct was cannulated under non-sterile conditions. The pancreas was distended by infusion of 10 ml sterile Krebs-Ringer-Hepes solution containing 10 % Bovine Serum Albumin (BSA fraction V, Sigma). Subsequently, the pancreas was excised and brought into a laminar flow cabinet. All further procedures were performed under sterile conditions.

The isolation procedure was identical for all four strains. In short, the donor pancreas was removed after infusion of 10 ml sterile Krebs-Ringer-Hepes solution containing 10 % Bovine Serum Albumin (BSA) through the bile duct. Excised pancreata were chopped and digested using a two-stage incubation of 20 min at 37°C with successively 1.0 and 0.7 mg/ml collagenase (P, Boehringer Mannheim). Islets were separated from exocrine tissue by centrifugation over a discontinuous Dextran (Sigma) gradient (17) and further purified by handpicking. Islets were cultured in non-treated petri-dishes (Greiner, Alphen a/d/ Rijn, The Netherlands) in portions of approximately 100 islets per 25 cm^2 in CMRL 1066 (Gibco), containing 10% fetal calf serum (FCS, Gibco), 8.3 mmol/l glucose, 10 mmol/l Hepes and 1% Penicillin/Streptomycin (Gibco), at 37 °C in humified air containing 5% CO_2.

Glucose challenge test

For each rat strain, six separate samples of 20 islets each were tested simultaneously for their stimulated insulin secretion. Islets (150 ± 50 μm in diameter) were first pre-incubated for 45-50 minutes in 2 ml Krebs-Ringer-Bicarbonate (KRB), containing 0.25 % BSA and 2.75 mM glucose, and gassed with 95 % O_2 and 5 % CO_2. Insulin secretion was then assessed by three consecutive incubations of 45 minutes in KRB containing (i) 2.75 mM glucose, (ii) 16.5 mM glucose, and (iii) 2.75 mM glucose. For three out of six samples,

the high glucose (16.5 mM) incubation step was performed with 0.1 % 3-isobutyl-1-methylxanthine (IBMX, Sigma). At the end of each incubation, KRB was completely removed and frozen for insulin determination by radioimmunoassay for rat insulin (Linco, Ede, the Netherlands). Insulin secretory responses of 20 islets during 45 minutes of incubation were expressed as nanograms of insulin/ml. To eliminate differences in insulin response due to variation in islet size (9), stimulation indices were calculated by dividing stimulated insulin secretion by basal insulin secretion.

Statistics

All data were analyzed by Mann Whitney U tests with a two-tailed distribution.

Results

Table 1 shows islet isolation yield per rat strain. AO rats donated almost twice the number of islets as compared to Lewis and Wistar rats ($P<0.10$). Islet purity (*i.e.* the number of islets free of exocrine tissue) was over 90% for AO, Lewis and Wistar rats after dextran gradient purification on day zero. Approximately half of the number of islets isolated from SD rats was in part still embedded in exocrine tissue after purification. For all strains, including SD, islet purity was 98 % - 100 % after two days of culture and after seven days of culture.

Table 2 shows the stimulated insulin secretion of islets after challenge with glucose together with or without IBMX. IBMX significantly increased the glucose stimulated insulin secretion of islets of all rat strains. Without IBMX the stimulated insulin secretion of SD islets on day 2 (5.7 ± 1.4) and on day 7 (6.5 ± 2.8) were not significantly elevated compared to basal insulin secretion

Table 1 *Number of islets (± sd) isolated per pancreas from Albino Oxford (AO), Lewis, Wistar and Sprague Dawley (SD) rats.* # *P<0.10 compared to Lewis and Wistar.* ¶ *P<0.001 compared to Wistar.*

	AO	Lewis	Wistar	SD
Present study	614 ± 253 #	303 ± 46	351 ± 72	346 ± 174
Previous studies	549 ± 111 ¶		288 ± 77	

TABLE 2 *Stimulated insulin secretion (ng/ml ± sem) of islets during a glucose challenge test with and without IBMX. * P<0.05 compared to the glucose stimulated insulin secretion after challenge without IBMX.*

		AO	Lewis	Wistar	SD
Day 0	- IBMX	12.7 ± 1.4	34.5 ± 8.8	22.9 ± 6.1	18.7 ± 3.0
	+ IBMX	56.5 ± 12.0*	79.6 ± 5.8*	87.0 ± 14.9*	64.7 ± 7.1*
Day 2	- IBMX	11.1 ± 3.2	10.1 ± 2.4	11.4 ± 1.5	5.7 ± 1.4
	+ IBMX	32.2 ± 9.0*	48.4 ± 5.9*	51.2 ± 2.2*	20.6 ± 1.3*
Day 7	- IBMX	12.6 ± 3.5	24.3 ± 8.6	13.1 ± 4.7	6.5 ± 2.8
	+ IBMX	69.7 ± 8.7*	66.9 ± 14.3 *	47.3 ± 12.3*	33.5 ± 12.6*

levels on the same day (3.6 ± 1.3 and 3.2 ± 1.1 respectively). The stimulated insulin secretion of AO islets on day two (11.1 ± 3.2) was highly (P<0.10) compared to basal insulin secretion on the same day (4.2 ± 0.8). Basal insulin secretion levels during the first phase of the glucose challenge test typically varied between 2.2 ng/ml and 6.3 ng/ml for the other rat strains. Stimulation indices, which were calculated by dividing stimulated insulin secretion by basal insulin secretion to reduce possible variation due to size, are shown in FIGURE 1 and FIGURE 2.

Few differences are apparent when comparing the stimulation indices after glucose challenge without IBMX (FIGURE 1). AO islets have an improved stimulation index on day 7 compared to day 0 and day 2. Lewis islets have a higher stimulation index on day 0 compared to day 2 and day 7. The stimulation indices of SD islets after 2 days and 7 days of culture are lower compared to day 0. Compared to AO and Wistar, Lewis islets showed the highest stimulation index on day 0. There were no differences in the secretory response between the rat strains after two days of culture. After seven days of culture, the stimulation index of AO islets was higher compared to Lewis and SD islets (P<0.05, TABLE 2). With IBMX (FIGURE 2), the stimulation indices of AO and SD islets are lower (P<0.05) after two days of culture compared to the stimulation indices of freshly isolated islets. SD islets had a stimulation index that was lower compared to Lewis islets after two days of culture.

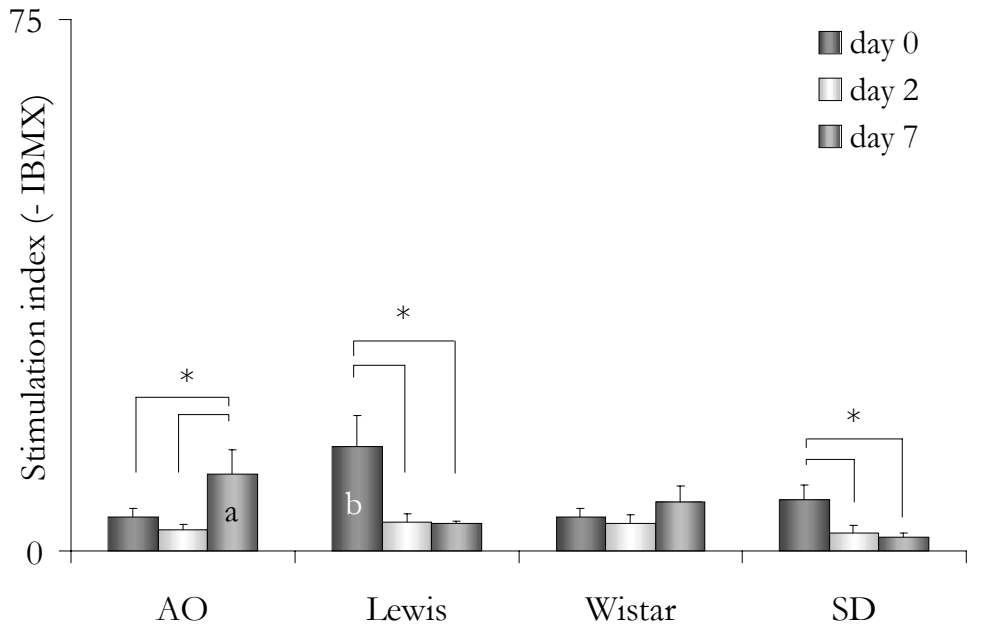

FIGURE 1. *Stimulation indices of Albino Oxford (AO), Lewis, Wistar and Sprague Dawley (SD) islets immediately after isolation (day 0), after short time culture (day 2) and after long time culture (day 7). Islets were stimulated with glucose only. (* = P<0.05; a denotes compared to Lewis and SD on day 7; b denotes compared to AO and Wistar on day 0).*

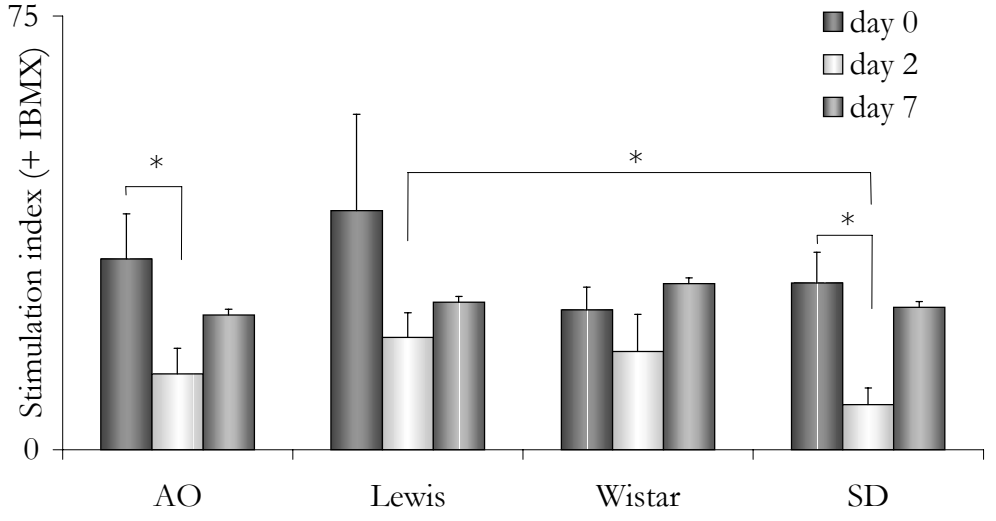

FIGURE 2. *Stimulation indices of Albino Oxford (AO), Lewis, Wistar and Sprague Dawley (SD) islets immediately after isolation (day 0), after short time culture (day 2) and after long time culture (day 7). Islets were stimulated with glucose in combination with 0.1% 3-isobutyl-1-methylxanthine (IBMX). (* = P<0.05).*

Discussion

Effective islet isolation is crucial for islet studies. Islet isolation yields, and islet function depend on many factors and may even be strain dependent. In the present study we compared four rat strains regarding islet yield and islet function and found them to be strain dependent. Our interest was based on previous results from our laboratory. We retrospectively analyzed the islet yields of several experiments where either AO rats or Wistar rats were used as donors. For these experiments, the islet isolation procedure was identical to the islet isolation procedure applied here. These analyses show that the islet yield of AO rats (549 ± 111, n=84) was significantly higher ($P<0.001$, two tailed Student t-test) compared to Wistar rats (288 ± 77, n=78). Results of the present study are in accordance with the retrospective analyses. AO rats donate almost twice the number of islets compared to Wistar, Lewis and SD rats. Due to the high variation in islet yield per isolation in the present study, this difference was significant only with a probability of $P<0.10$ compared to Wistar and Lewis. The somewhat higher number of isolated islets per rat strain in the present study (614 ± 253 for AO and 303 ± 46 for Wistar) compared to the retrospectively analyzed studies can be attributed to the fact that more animals were isolated at the same time during the previous studies. This leads to longer procurement time of the organs, which is associated with lower islet yields (5, 14). Not only islet yield, but also islet purity differed among the rat strains. A highly purified islet mass could be isolated from AO, Lewis and Wistar rats, but not from SD rats. Thus, islet isolation yield and islet purity are rat strain dependent. Using AO rats instead of Lewis, Wistar or SD can halve the number of animals necessary for experimental studies.

The secretory response to glucose with IBMX is higher compared to glucose without IBMX for all rat strains and all conditions, except for SD islets after short time culture. We found a 5-fold increase of the stimulation index (average of all conditions), which is in line with the 5.7-fold increase observed by Wiedenkeller and Sharp (20). The low stimulation index of SD islets after culture illustrates an inferior islet function rather than an ineffectiveness of IBMX. Without IBMX, a high level of glucose could not increase the insulin secretion level of SD islets compared to a low level of glucose. For AO islets after two days of culture, glucose stimulation only slightly increased insulin secretion compared to basal insulin secretion ($P<0.10$). Thus, for glucose challenge IBMX is necessary to guarantee a significant secretory response to glucose after both short and long time culture of SD islets and after short culture of AO islets. The stimulation index of Lewis islets after glucose challenge without IBMX is almost three times as high as compared to AO and Wistar islets. This implies that, with regard to islet function, Lewis rats are preferential as islet donors above AO and

Wistar rats for studies where high secretory responses are required immediately after isolation.

In summary, rat islet function, islet purity, and islet isolation yield are donor-strain dependent. Without IBMX, Lewis islets have superior secretory responses immediately after islet isolation compared to the other rat strains, which is an argument in favor of Lewis rats as islet donors. IBMX significantly enhances the insulin secretion during glucose challenge, which improves the islet function test. For *in vitro* purposes we suggest that AO rats are the optimal donor source, since the number of experimental animals can be reduced twofold compared to Lewis, Wistar or SD rats. AO islets function excellent during a one week culture period, and for best secretory responses after short time culture IBMX is recommended.

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