Pancreatic islet function in a transgenic mouse expressing fluorescent protein

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Abstract

Pancreatic islet function and glucose homeostasis have been characterized in the transgenic YC-3·0 mouse, which expresses the yellow chameleon 3·0 (YC-3·0) protein under the control of the β -actin and the cytomegalovirus promoters. Fluorescence from the enhanced yellow fluorescent protein (EYFP), one part of the yellow chameleon protein, was used as a reporter of transgene expression. EYFP was expressed in different quantities throughout most cell types, including islet endocrine and stromal cells. No adverse effects of the transgene on animal health, growth or fertility were observed. Likewise, *in vivo* glucose homeostasis, mean arterial blood pressure and

regional blood flow values were normal. Furthermore, the transgenic YC-3·0 mouse had a normal β -cell volume and mass as well as glucose-stimulated insulin release *in vitro*, compared with the C57BL/6 control mouse. Isolated islets from YC-3·0 animals continuously expressed the transgene and reversed hyperglycemia when transplanted under the renal capsule of alloxan-diabetic nude mice. We conclude that isolated pancreatic islets from YC-3·0 animals implanted into recipients without any EYFP expression, constitute a novel and versatile model for studies of islet engraftment.

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Introduction

The green fluorescent protein (GFP) from the jellyfish, *Aequorea victoria*, and its derivatives are today used in numerous hybrid proteins as cellular reporters. Ever since the first use of the wild-type GFP as an intracellular reporter (Chalfie *et al.* 1994), the GFP molecule has been modified to generate variants with enhanced fluorescent signals (Heim *et al.* 1995, Scholz *et al.* 2000), and altered excitation and emission properties, providing new fluorescence colors such as blue, cyan and yellow (Heim *et al.* 1994, Ormö *et al.* 1996). In combination with fluorescence microscopy techniques, these mutated GFP variants have facilitated live studies of gene expression and protein localization, not only in cell lines but also in transgenic mice (Okabe *et al.* 1997, Leibiger *et al.* 1998, Hadjantonakis *et al.* 2002). The ability to generate transgenic mice expressing fluorescent reporters make them a valuable tool, not only for studies of the animals *per se*, but also as donors for studies of cell and organ transplantation (Hess *et al.* 2003). Transplantation of fluorescent cells into

a non-fluorescent host provides a direct method to both identify and monitor the donated tissue over time, either *in vivo* in the host or in tissue preparations.

Recently, the introduction of the 'Edmonton protocol' has substantially increased the success rate of clinical islet transplantations (Shapiro *et al.* 2000). Although the clinical results are encouraging, increased knowledge about the function of pancreatic islets following transplantation is required to establish islet transplantation as a treatment for type 1 diabetes. To facilitate future structural and functional studies of transplanted islets, we have investigated glucose homeostasis and function of the pancreatic islets in the transgenic YC-3·0 mouse. The YC-3·0 mouse is characterized by expression of the yellow chameleon 3·0 (YC-3·0) protein under regulation of both the β -actin and the cytomegalovirus (CMV) promoters. Dual promoters were used to ensure a ubiquitous expression. Fluorescence from the enhanced yellow fluorescent protein (EYFP), one part of the hybrid YC-3·0 protein, was used as a reporter for the transgene and was found in different quantities throughout most tissues, including the

islet endocrine and stromal cells. The transgenic YC-3·0 mouse represents, with its pancreatic islet EYFP expression and normal islet and β -cell function, a new and valuable tool for studies of islet engraftment and hormone secretion from transplanted β -cells.

Materials and Methods

Animals

The YC-3·0 transgenic mouse, kindly donated by Professor R Y Tsien at the University of California, has been described previously (Tsai *et al.* 2003). Briefly, the gene encoding the yellow chameleon protein-3·0 (Miyawaki *et al.* 1997) along with the β -actin and CMV promoters were introduced into fertilized eggs from a 129J $\times$ C57BL/6J background. The founders were backcrossed once with 129J, before they were propagated by brother-sister mating. Homozygous animals were then identified and used for the establishment of the colony. YC-3·0 mice of both genders, except when stated otherwise, between 2 and 9 months of age were used for islet isolation and autopsy. YC-3·0 mice between 4 and 6 months of age were used for *in vivo* studies. Male C57BL/6 and C57BL/6 nude mice weighing 25–30 g were purchased from the B&M Research and Breeding Center (Ry, Denmark) and were subsequently used as control animals. All animals had free access to tap water and pelleted food and were housed in a room with a 12 h light/12 h darkness cycle and humidity of 70% throughout the course of the study. All experiments were approved by the local animal ethics committees at Uppsala University and Karolinska Institutet.

Studies of EYFP fluorescence in different tissues

For studies of whole animal fluorescence, animals were killed and the skin together with the underlying muscle layer was removed from the abdominal side. Pictures of whole animal fluorescence were captured using a cooled CCD camera (Astrocam TE3/A/S with Site 502AB-1; PerkinElmer Life Sciences, Cambridge, Cambs, England) connected to a Leica MZFLIII stereomicroscope with filters for EYFP fluorescence (Leica Microscopy Systems Ltd, Heerbrugg, Switzerland). Using the described equipment and living animals under continuous inhalation anesthesia (Isoflurane; Abott Scandinavia AB, Solna, Sweden), EYFP fluorescence from transplanted YC-3·0 islets beneath the renal capsule was visualized after moving the overlying organs. The intensity of EYFP fluorescence in different tissues was measured in live organs after excision from sacrificed animals. The organs were examined using a Leica TCS-SP2-AOBS confocal laser-scanner connected to a Leica DMLFSA microscope (Leica Microsystems Heidelberg GmbH, Mannheim, Germany).

Optical sections were captured using a Leica HCX APOL 40 $\times$ /0·80 W objective and excitation light at 514 nm with detection of emission light between 516 and 600 nm.

Preparation of histological sections and staining procedures

For morphological investigations, organs were fixed in 10% (vol/vol) neutral buffered formalin and embedded in paraffin. Sections, 5 μ m thick, were cut and mounted on glass slides and stained with hematoxylin-eosin or an antibody against insulin, as previously described (Mattsson *et al.* 2002). Briefly, the sections were deparaffinized, washed in Tris-buffer solution (TBS), blocked with hydrogen peroxide and serum, and then incubated with a primary antibody against insulin raised in guinea pig (ICN Biomedicals Inc., Aurora, OH, USA). Thereafter, the sections were washed, covered with secondary goat anti-guinea pig antibody, and finally developed with 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma-Aldrich, St Louis, MO, USA). The sections were counterstained with hematoxylin and a coverslip was mounted. The visualization of the islets in these samples enabled us to estimate the volume of the islets within the pancreatic regions, and thereby also the islet mass, by a point-counting method previously described in detail (Carlsson *et al.* 1996).

For immunofluorescence, pieces of pancreas were fixed in 4% (vol/vol) neutral buffered formaldehyde for 4 h, washed in PBS and transferred to 18% sucrose for 2 h before incubation overnight in 30% sucrose at 4 °C. The tissue was embedded in Tissue-Tek O.C.T. Compound (Sakura Finetek, Zoeterwoude, The Netherlands), frozen on dry ice and stored at -80 °C. Tissue sections of 10 μ m were cut using a cryostat (Leica CM 3000; Leica Microsystems, Nussloch, Germany) and adhered to glass slides (SuperfrostPlus; Menzel, Braunsweig, Germany). The sections were washed with OptiMax wash buffer (Biogenex, San Ramon, CA, USA) before blocking with Universal Blocker Reagent (Biogenex) for 5–10 min and goat serum for 20 min (Biogenex). Primary antibodies were applied for 1 h, using a ready-to-use polyclonal guinea pig anti-insulin antibody (Biogenex), a ready-to-use polyclonal rabbit anti-glucagon antibody (Biogenex) and a monoclonal rat anti-mouse CD31 (PECAM-1) antibody (BD Bioscience Pharmingen, San Diego, CA, USA) at 1:50 dilution in PBS. The sections were washed before the secondary antibodies, Alexa Fluor 633 anti-guinea pig, anti-rabbit or anti-rat (Molecular Probes, Eugene, OR, USA), were applied at 1:200 dilution in PBS for 20 min. Finally, the sections were washed and mounted with coverslips using ProLong Antifade (Molecular Probes). The sections were scanned using the described Leica TCS-SP2-AOBS confocal laser-scanner with a Leica HCX PL APO CS 63 $\times$ /1·2 W objective. Images were captured using sequential scanning, first with 514 nm as excitation light and detection of emission light

between 518 and 560 nm for EYFP and secondly with 633 nm as excitation light and detection of emission light between 645 and 680 nm for Alexa Fluor 633.

Islet isolation and culture

Pancreatic islets were isolated by a collagenase digestion method, as previously described (Andersson 1978). The islets were cultured free-floating in an incubator at 37 °C with 5% CO₂ in air, with 150 islets in each culture dish, in 5 ml culture medium, RPMI 1640 (Sigma-Aldrich) supplemented with L-glutamine (Sigma-Aldrich), benzylpenicillin (100 U/ml; Roche Diagnostics Scandinavia, Bromma, Sweden), streptomycin (0.1 mg/ml; Sigma-Aldrich) and 10% (vol/vol) fetal calf serum (Sigma-Aldrich). The medium was changed every second day.

Islet transplantation

Fed male C57BL/6 nude mice were injected intravenously with alloxan (80 mg/kg body weight; Sigma-Aldrich). Blood glucose concentrations were measured with test reagent strips one week after the injections and animals with concentrations exceeding 18 mmol/l were considered diabetic. These animals were anesthetized with an intraperitoneal injection of 0.02 ml/g body weight of avertin, a 2.5% (vol/vol) solution of 10 g 97% 2,2,2-tribromo-ethanol (Sigma-Aldrich) in 10 ml 2-methyl-2-butanol (Kemila, Stockholm, Sweden). The left kidney was exposed through a flank incision and 400 islets, isolated (see above) from YC-3.0 mice and cultured for 3–4 days, were implanted under the renal capsule. Blood glucose concentrations were then measured weekly for 4 weeks. Thereafter, the mice were killed and the grafts were removed, fixed in formalin, embedded in paraffin and stained with antibodies for insulin (ICN Biomedicals Inc.) as previously described in detail (Mattsson *et al.* 2002).

Glucose-stimulated insulin release

Groups of ten islets were transferred in triplicates to glass vials containing 250 µl Krebs-Ringer bicarbonate buffer supplemented with 10 mM HEPES (Sigma-Aldrich) and 2 mg/ml bovine serum albumin (ICN Biomedicals Inc.) (hereafter referred to as KRBH buffer). The KRBH buffer contained 1.67 mM D-glucose during the first hour of incubation at 37 °C (O₂:CO₂, 95:5). The medium was removed and replaced by 250 µl KRBH supplemented with 16.7 mM glucose and incubated for a second hour. After retrieval of the media, the islets were harvested, pooled in groups of 30, and homogenized by sonication in 200 µl redistilled water. Two 50 µl aliquots of the aqueous homogenate were used for DNA measurements by fluorophotometry (Hinegardner 1971). A fraction of the homogenate was mixed with acid-ethanol (0.18 M HCl in

95% (vol/vol) ethanol) from which insulin was extracted overnight at 4 °C. Insulin concentrations in incubation media and homogenates were determined by a commercial mouse insulin ELISA (Mercodia, Uppsala, Sweden).

Glucose oxidation rate

Islet glucose oxidation rates were determined according to a previously described method (Andersson & Sandler 1983). Briefly, triplicates of ten islets were transferred to glass vials containing 100 µl KRBH supplemented with D-[U-¹⁴C]glucose (Amersham-Pharmacia Biotech, Amersham, Bucks, UK) and non-radioactive D-glucose to a final glucose concentration of 16.7 mM (specific radioactivity 0.5 mCi/mM) and the produced ¹⁴CO₂ was captured with hyoscyamine. After incubation for 90 min at 37 °C (O₂:CO₂, 95:5), the oxidation was terminated by injection of 100 µl 0.05 mM antimycin A (Sigma-Aldrich) into the vials. The ¹⁴CO₂ generated by cell metabolism was released by the addition of 100 µl 0.4 mM NaH₂PO₄ (pH 6.0) during 120 min incubation. The radioactivity in the samples was then measured by liquid scintillation counting.

Pancreas perfusions

The mice were anesthetized with an intraperitoneal injection of 0.02 ml/g body weight of avertin (see above). The pancreas and duodenum were then prepared for perfusion according to a previously described technique (Jansson 1986). Briefly, the pancreas and duodenum were carefully dissected and removed, together with the section of the aorta from which the celiac and superior mesenteric arteries emanate. The aorta and the portal vein were catheterized and a catheter was inserted into the duodenum to divert the exocrine secretions from the pancreas and the intestine. The preparation was then transferred to a perfusion chamber where temperature (37 °C) and humidity (100%) were kept constant. Perfusion was performed with KRBH supplemented with 2% dextran T70 (Pharmacia-Upjohn, Uppsala, Sweden). Either 2.8 or 16.7 mmol/l D-glucose or 5.5 mmol/l D-glucose + 10 mmol/l L-arginine (Sigma-Aldrich) were added to the KRBH. Perfusion was kept at a constant flow rate of 1 ml/min and the medium was continuously gassed with oxygen (O₂:CO₂, 95:5) and kept at a temperature of 37 °C. The experiments started with perfusion with 2.8 mmol/l glucose (15 min), followed by 16.7 mmol/l glucose (20 min), 2.8 mmol/l glucose (15 min), 5.5 mmol/l glucose + 10 mmol/l arginine (10 min) and finally 2.8 mmol/l glucose (15 min). Aliquots (1 ml) of the effluent fluid were collected from the portal vein and stored at –20 °C. The insulin concentrations in these samples were measured with ELISA (Mercodia). Any experiments, i.e. two experiments, where the insulin concentrations did not return to basal values during

perfusion with low glucose (2·8 mmol/l) concentrations were excluded from the study.

Blood flow measurements

The experiments were performed according to a protocol previously described in detail (Carlsson *et al.* 1997). Briefly, non-fasted male mice were anesthetized with an intraperitoneal injection of avertin (see above), heparinized and placed on a heated operating table. Polyethylene catheters were inserted via the right carotid artery into the ascending aorta and into the femoral artery. The cranial catheter was connected to a pressure transducer (PDCR 75/1; Druck Ltd, Groby, Leics, UK), thereby allowing constant monitoring of the mean arterial blood pressure. After a stable blood pressure was achieved, $1\cdot5\text{--}2\cdot0\times 10^5$ non-radioactive microspheres (EZ-Trac; DuPont Pharmaceuticals Inc., Wilmington, DE, USA) with a mean diameter of 11 μm were injected during 10 s via the catheter placed with its tip in the ascending aorta. Starting 5 s before the microsphere injection and continuing for a total of 60 s, an arterial blood sample was collected from the catheter in the femoral artery at a rate of approximately 0·20 ml/min. The exact withdrawal rate was confirmed in each animal by weighing the sample. After obtaining the reference sample, another blood sample was drawn for measurement of blood glucose. After the animals had been killed, the whole pancreas and both adrenal glands as well as samples from the duodenum, colon and left kidney were removed, blotted and weighed. The tissue samples were then treated with a freeze-thawing technique in order to visualize the microspheres as previously described (Jansson & Hellerström 1981). The blood flow values were calculated according to the formula $Q_{\text{org}} = Q_{\text{ref}} \times N_{\text{org}}/N_{\text{ref}}$ where Q_{org} is organ blood flow (ml/min), Q_{ref} is withdrawal rate of the reference sample (ml/min), N_{org} is number of microspheres present in the organ and N_{ref} is number of microspheres in the reference sample. A difference of <10% in blood flow values between the adrenal glands was used to confirm adequate mixing of the spheres in the circulation.

Statistical analysis

All values are given as means $\pm$ S.E.M. Student's unpaired *t*-test was used and $P<0\cdot05$ was considered to be statistically significant for all comparisons.

Results

Animals transgenic for YC-3·0 grew normally, and showed no external or internal signs of malformations or any other adverse reactions to the transgene.

Organ morphology

The majority of organs were normal in macroscopic appearance and size. Examination of hematoxylin-eosin

Table 1 Organ weights, blood glucose, serum insulin concentrations and blood flow values in anesthetized male C57BL/6 and YC-3·0 mice. Values are means $\pm$ S.E.M.

	C57BL/6 (n=10)	YC-3·0 (n=11)
Body weight (g)	27·8 $\pm$ 0·3	30·6 $\pm$ 1·5
Pancreas weight (mg)	314 $\pm$ 4	300 $\pm$ 8
Heart weight (mg)	434 $\pm$ 9	446 $\pm$ 9
Adrenal weight (mg)	4·4 $\pm$ 0·2	5·8 $\pm$ 0·4*
Blood glucose (mmol/l)	8·8 $\pm$ 0·9	7·6 $\pm$ 0·3
Serum insulin (ng/ml)	1·69 $\pm$ 0·28	2·03 $\pm$ 0·30
Duodenal blood flow (ml/min $\times$ g)	4·31 $\pm$ 0·73	5·02 $\pm$ 0·98
Colonic blood flow (ml/min $\times$ g)	2·30 $\pm$ 0·27	1·97 $\pm$ 0·36
Renal blood flow (mg/min $\times$ g)	2·93 $\pm$ 0·42	3·51 $\pm$ 0·69
Adrenal blood flow (ml/min $\times$ g)	13·38 $\pm$ 2·14	18·06 $\pm$ 2·44

* $P<0\cdot02$ when calculated with Student's unpaired *t*-test.

stained sections from the heart, small intestine, kidney and liver demonstrated no abnormalities in the transgenic mice (data not shown). One notable finding was that the adrenal glands were significantly heavier in YC-3·0 mice than in C57BL/6 mice (Table 1). However, the morphology of the glands was normal.

Tissue expression of EYFP

The intensities of EYFP fluorescence in different tissues varied markedly (Fig. 1). Strong EYFP fluorescence was seen in the exocrine pancreas and in muscle tissue in all animals, whereas the fluorescence was weaker in other organs. In some organs, such as kidney and liver, the intensity of fluorescence varied between different groups of cells ($n=2$, data not shown). Morphological investigations of fixed cryosections from the YC-3·0 pancreas, comparing EYFP fluorescence to pancreatic islet morphology under phase contrast conditions, showed that the transgene was expressed in all pancreatic islet cells (30 islets in 10 different sections from 3 animals, data not shown). Immunohistochemical staining using antibodies against insulin, glucagon and CD31 further verified that antibody labeled β -, α - and endothelial cells expressed the transgene (Fig. 2). Isolated pancreatic islets from YC-3·0 mice were fluorescent and could easily be distinguished compared with islets from C57BL/6 mice (Fig. 3). The YC-3·0 islets retained their EYFP fluorescence after transplantation and engraftment in athymic nude mice. The EYFP fluorescence from the graft was easily detected 6 months after transplantation (Fig. 4).

Pancreatic morphology

The weight of the pancreas was similar in the C57BL/6 and the YC-3·0 mice (Table 1). The strong expression of YC-3·0 in the exocrine pancreas provided a distinct yellowish color. The light microscopic morphology of both

Figure 1 Whole animal EYFP fluorescence in a YC-3.0 transgenic mouse and a C57BL/6 mouse is shown. Using identical instrumental settings for the pictures of the corresponding conditions of the two animals, pictures were captured with white light (A and D) and with fluorescence using filters for EYFP fluorescence (B and E). To illustrate the difference in intensity level between the EYFP fluorescence in the YC-3.0 mouse and the autofluorescence in the C57BL/6 mouse, the lower intensities were enhanced (in C and F), as illustrated by the scale.

the islets and the exocrine pancreas in YC-3.0 mice was normal, and could not be distinguished from that of C57BL/6 mice. Immunostaining for insulin demonstrated a normal distribution of β -cells within the islets of YC-3.0 mice and did not differ from that of C57BL/6 mice. Islet volume (0.66 ± 0.07 vs $0.80 \pm 0.06\%$ in C57BL/6 ($n=10$) and YC-3.0 ($n=11$) mice respectively) and islet mass (2.12 ± 0.23 vs 2.69 ± 0.18 mg in C57BL/6 ($n=10$) and YC-3.0 ($n=11$) mice respectively) were similar in the two strains of mice. There were no signs of insulinitis or any other type of inflammatory process in the transgenic mice.

In vitro studies of islets

The islet DNA content of C57BL/6 and YC-3.0 mice was similar (21.5 ± 0.6 vs 20.8 ± 0.2 $\mu\text{g}/10$ islets respectively; $n=5$ in each group). Also the insulin content was similar when the two strains of mice were compared (0.29 ± 0.04 vs 0.33 ± 0.04 ng/10 islets respectively; $n=5$ in each group). Insulin release in response to 1.67 or 16.7 mM glucose was investigated after 3–4 days of culture

(Fig. 5A). Both strains responded with a pronounced increase in insulin release at the higher glucose concentration, but no differences between C57BL/6 and YC-3.0 mice were detected at either the low or the high glucose concentrations (Fig. 5A). When exposed to 16.7 mM glucose, glucose oxidation rates of islets from C57BL/6 and YC-3.0 mice were similar (285 ± 45 vs 359 ± 34 pmol/10 islets $\times$ 90 min; $n=7$ in each group). Likewise, pancreata of both strains showed a pronounced biphasic insulin release of a similar magnitude in response to both 16.7 mmol/l glucose and 10 mmol/l L-arginine (Fig. 5B).

Glucose tolerance in vivo

In intravenous glucose tolerance tests YC-3.0 and C57BL/6 mice showed similar blood glucose curves (Fig. 6). Furthermore, YC-3.0 mice were sensitive to the diabetogenic effects of alloxan, but the dose needed to induce permanent hyperglycemia was slightly higher

Figure 2 Confocal images of three islets from sections of YC-3·0 pancreas are shown. The upper panel shows the transgene EYFP fluorescence in one section from each islet (A-C). The lower panel shows the Alexa 633 fluorescence after antibody staining against insulin (D), glucagon (E) and CD-31 (F) in the same sections of the corresponding islets. The shown pictures are representative of 7 islets of each staining from 2 animals. The scale bars correspond to the respective sections and represent 20 µm.

(80 mg/kg) than in C57BL/6 mice (70 mg/kg; data not shown).

Figure 3 Optical sections from the center of isolated pancreatic islets from a YC-3·0 mouse (A and B) and a C57BL/6 mouse (C and D) are shown. The pictures of each islet are captured simultaneously with transmission light and EYFP fluorescence excitation light at 514 nm, using identical settings for the YC-3·0 and the C57BL/6 islet. The shown pictures are representative of 6 islets isolated from 3 animals of each condition. The scale bars represent 40 µm.

Transplantations with islets from YC-3·0 mice

A total of 5 alloxan-diabetic nude mice were implanted with 400 islets isolated from YC-3·0 mice. Their blood glucose concentrations decreased from 23.4 ± 3.2 mmol/l

Figure 4 The pictures show the left kidney of an anesthetized athymic nude mouse. A graft from 200 transplanted YC-3·0 pancreatic islets can be seen beneath the kidney capsule. Keeping the microscope in a fixed position, pictures were captured during white light (left panel) and during fluorescence using filters for EYFP fluorescence (right panel). The shown pictures are representative of 3 investigated grafts.

Figure 5 (A) Insulin release from isolated islets cultured for 3–4 days before the experiments and then exposed to 1.67 or 16.7 mmol/l D-glucose. Islets were derived from C57BL/6 or YC-3.0 mice. Values are means $\pm$ S.E.M. of 6 experiments. (B) Insulin concentrations in the effluent media collected from the perfused pancreas-duodenum from C57BL/6 mice (solid symbols) and YC-3.0 mice (open symbols). The insulin release was stimulated with 16.7 mmol/l D-glucose (15–35 min) and with 5.5 mmol/l D-glucose + 10 mmol/l L-arginine (50–60 min). At all other time points the organs were perfused with KRBH buffer containing 2.8 mmol/l D-glucose. Values are given as means $\pm$ S.E.M. of 5–6 animals.

($n=5$) to below 10 mmol/l after 1, 2 and 4 weeks (8.8 ± 1.0 , 7.9 ± 0.7 and 7.8 ± 1.1 mmol/l respectively). A general morphological evaluation of the islet grafts showed a normal morphology of rounded islet-like structures with an interspersed vascular stroma. The β -cell was the dominant cell type in the islet grafts and constituted approximately 66–75% of the grafted cells (data not shown).

In vivo studies on the circulation

The mean arterial blood pressure (78 ± 3 vs 73 ± 2 mmHg respectively) as well as the hematocrit (43 ± 1 vs $44 \pm 1\%$

Figure 6 Blood glucose concentrations at different times after an intravenous injection of 250 μ l 30% glucose solution in C57BL/6 (solid symbols) or YC-3.0 (open symbols) mice. Values are means $\pm$ S.E.M. of 5–6 experiments.

respectively) were similar in C57BL/6 ($n=10$) and YC-3.0 ($n=11$) mice. Blood glucose and serum insulin concentrations were similar in the two strains of mice under anesthesia at the time of the blood flow measurements (Table 1), as was the ratio between heart and body weight (1.5 ± 0.2 ($n=10$) vs $1.4 \pm 0.2\%$ ($n=11$) respectively). Total pancreatic blood flow (1.11 ± 0.12 vs 1.22 ± 0.12 ml/min $\times$ g pancreas) and islet blood flow (7.0 ± 1.3 vs 11.1 ± 2.9 μ l/min $\times$ g pancreas) did not differ in male C57BL/6 ($n=10$) and YC-3.0 ($n=11$) mice respectively. No differences in any of the other investigated blood flow values were seen (Table 1).

Discussion

The present study describes the physiology, with special emphasis on the pancreatic islets, in the transgenic YC-3.0 mouse. The YC-3.0 strain is characterized by a transgene expression of the YC-3.0 protein, including the EYFP, under the control of the β -actin and the CMV promoters. We have used EYFP fluorescence to measure the transgenic expression in different tissues and found a mosaic expression pattern. This finding is consistent with previous observations from the YC-3.0 mouse (Tsai *et al.* 2003), and the observed strong expression in the exocrine pancreas and the muscle tissue correlates with previous experiences utilizing the CMV promoter (Zhan *et al.* 2000). More importantly, we have shown that the transgene has a strong expression that is easily detected by EYFP fluorescence throughout all pancreatic islet cells. During this study, no attempt was made to use the YC-3.0 protein as a Ca^{2+} indicator.

A careful mapping of pancreatic islets from the YC-3.0 mouse showed that they were functionally similar to islets

from the C57BL/6 mouse with regard to size, insulin content, insulin release and glucose oxidation in response to hyperglycemia. The similarities in islet function *in vitro* between the two strains were supported by comparison of *in vivo* glucose tolerance tests from YC-3.0 and C57BL/6 mice, which showed similar patterns of glucose-stimulated insulin release and glucose clearance. The morphology of the pancreas and the islets in the YC-3.0 mouse was normal and indistinguishable from the morphology of the C57BL/6 mouse. Furthermore, mean arterial blood pressure and regional blood flow values were similar in both strains of mice. This is of particular interest since the transgene was under the control of the β -actin promoter and was found to be expressed in, for example, the myocardium. Previous studies have demonstrated that the closely related GFP may induce myocarditis when expressed in high quantities in the heart (Huang *et al.* 2000). The absence of such signs of abnormalities in the YC-3.0 mouse was further supported by the normal ratio between heart weight and body weight. Thus, all data both *in vivo* and *in vitro*, suggest that the YC-3.0 mouse has normal physiology. The only difference noted between the YC-3.0 and the C57BL/6 mice was that the adrenal glands were heavier in the former strain. However, the morphology of the glands was normal and since the YC-3.0 mouse is not fully backcrossed to a C57BL/6 background, this difference in organ size may be the result of strain variance. In addition, reversal of hyperglycemia after transplantation of YC-3.0 islets to athymic mice with alloxan-induced diabetes, further supported the notion of a correct physiological function of the YC-3.0 islets.

By the generation of a transgenic mouse, a protein can be expressed at a persistent and known expression level. However, transgenic expression of protein constructs in living cells or organisms might interfere with endogenous DNA or protein function, resulting in altered cellular and whole organism physiology (Bertera *et al.* 2003). Thus, careful physiological examination of the cell or organism targeted for the transgenic expression is needed to exclude any unwanted side effects of the transgene. According to our results, the YC-3.0 mouse demonstrates the feasibility of constructing physiologically unaltered transgenic animals expressing fluorescent protein constructs throughout the pancreatic islet cells. This opens up the possibility of future approaches utilizing fluorescent protein constructs in order to directly target specific intracellular signaling pathways in islet cells. Recently, Hara *et al.* (2003), reported a transgenic mouse with a β -cell-specific GFP expression directed by the mouse insulin promoter (MIP-GFP), suggesting that this mouse would be a useful tool for various studies of β -cell function. These authors have also showed the usefulness of this mouse model for studies of transplanted β -cells (Hara *et al.* 2004). The YC-3.0 mouse and the MIP-GFP mouse represent two complementary models for studies of transplanted islets utilizing fluorescent techniques. In the YC-3.0 mouse, the β -actin- and

CMV-directed expression of the fluorescent reporter not only targets the expression to all islet cell types but also creates an expression that is independent of the metabolic functional status of the transplanted cells. In the MIP-GFP mouse, the insulin-promoter targets the expression of the fluorescent reporter specifically to β -cells and the expression is correlated to the β -cell's capacity to synthesize insulin, a capacity that might fail after transplantation into a diabetic recipient. These differences in localization and regulation of the two fluorescent reporters means that islets isolated from the YC-3.0 and the MIP-GFP mice serve as novel and complementary tools for studies of the islet function following transplantation.

Since the endothelial cells were fluorescent in islets derived from the YC-3.0 mouse, these islets will be useful in islet graft vascularization studies. We have recently shown that the vascular density in the islet graft is higher in the connective tissue stroma surrounding the islet cells than within the areas of endocrine cells (Mattsson *et al.* 2002). The use of the YC-3.0 mouse as an islet donor will enable us to evaluate to what extent the newly formed blood vessels in the islet graft derive from the donor or the recipient tissue and whether the stromal and the endocrine capillaries differ in this respect.

In conclusion, the transgenic YC-3.0 mouse offers a new source of well functional pancreatic islet cells endogenously labeled with EYFP. This source of fluorescent cells opens up new approaches to investigate transplanted islets, facilitating graft localization, as well as studies of graft morphology and function in both normoglycemic and hyperglycemic recipients.

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