ESM Methods

Islet isolation from 7-day-old Wistar or GK rats for gene analysis

This procedure was originally developed for adult pancreas [1] and adapted to neonates. Neonates were killed and the peritoneal cavity opened. Before to be excised, pancreata were inflated by occluding the duodenal extremity of the biliary duct with a clamp and injecting in the bile duct close to liver about 0.5 ml of an ice-cold collagenase solution prepared extemporaneously and consisting of 3.75 mg collagenase (lyophilized EC 3.4.24.3 from Clostridium histolyticum, type P, specific activity 3.24; Roche Diagnostics GmbH, Mannheim, Germany) in 7.5 ml Hank’s balanced salt solution (HBSS, BioWhittaker, Lonza, Verviers, Belgique) for 10 pancreata. Eight to thirteen inflated pancreata were pooled in a BD Falcon™ 50-ml conical tube (Becton Dickinson, Le Pont-de-Claiix, France) and incubated with the remaining collagenase solution for 17 min at 37°C. At the end of the incubation period, cold HBSS was added to bring to twice the volume of the initially prepared collagenase solution and collagenase digestion was achieved by a vigorous manual shaking for about 30 sec at room temperature. The resulting tissue digest was then transferred into a 150-ml glass container and washed 3-4 times with cold HBSS before to be passed through a 400 µm mesh nylon filter (Nytal, Heiden, Switzerland). The filtrate was again washed with about 100 ml cold HBSS and aliquots (2-5 ml) of the final preparation were transferred to Petri dishes and diluted with cold HBSS for collection of the islets one by one by hand-picking under a stereomicroscope. The islets were collected in cold HBSS containing 5.5 mM D-glucose and supplemented with 5 mg/ml of bovine serum albumin (BSA, fraction V fatty acid free; Roche Diagnostics, Germany), two consecutive collections being performed to minimize exocrine contamination. This procedure allowed to obtain 54±7 and 40±7 islets per pancreas from Wistar and GK neonates, respectively (n = 10-12 islet preparations). Finally, batches of 300-500 freshly isolated islets were collected in sterile 2-ml round bottom tubes and rapidly washed twice with cold HBSS. After centrifugation (2 min at 250 g) and removal of supernatant, islets were lysed by adding 600 µl of RLT lysis buffer (Qiagen, Hilden, Germany) supplemented with 1% (vol/vol) β-mercaptoethanol. Then, islet lysates were applied to a QIAshredder™ mini spin column, centrifuged for 2 mn at 1700 g and kept dry at –80°C until RNA extraction.