Suppl 2. GLUT2 protein and mRNA expression in brain regions of STZ-icv treated rats measured 1 month following the icv administration of 3 mg/kg STZ dose

**Animals**
Male Wistar rats (three-month-old) weighing 280-350 g (Department of Pharmacology, University of Zagreb School of Medicine) were used in the separate experiment. The rats were kept 2-3 per cage in a room with a 12 h light/12 h dark cycle (lights on 07:00 – 19:00 h), and the room temperature and humidity set in the range of 21-23°C and 40-70% respectively. All animals were kept on standardized food pellets and water *ad libitum*.

**Drug treatments**
Intracerebroventricular (icv) treatment was performed as described in the main text (Material and methods) with streptozotocin (STZ) given in two doses, on the first and third day (each time 1.5 mg/kg, dissolved in vehicle: 0.05 M citrate buffer, pH 4.5, bilaterally 2 μl/ventricle) to rats subjected to deep anesthesia (thiopental 40 mg/kg; 4 mg/kg diazepam). The total STZ dose was 3 mg/kg. Control (CTR) animals were given an equal volume of vehicle by the same procedure on the first and third day. There were 6 animals per each group (CTR and STZ), all together 12 animals.

**Tissue preparation**
Animals were sacrificed in deep anesthesia (thiopental 50 mg/kg; 5 mg/kg diazepam) 1 month following the treatment. After decapitation brains were quickly removed, with hippocampus (HPC), temporal cortex (TC) and parietal cortex (PC) being dissected out and frozen in liquid nitrogen. One side of the brain was used for Western blot analysis for glucose transporter 2 (GLUT2) by a procedure described in the main text (Material and methods) and the other side was used for real-time PCR analysis.

**Real-time RT-PCR - methodology**
Total RNA was isolated from each area of each rat brain region following the manual of a TRIRizol®Plus RNA Purification Kit (Thermo Fisher Scientific, Foster City, CA, USA), and RNA quantity was determined by OD A260/A280. The experiment for relative quantification of gene expression was performed on an Applied Biosystems 7500 Real-Time PCR System using *Relative Quantitation Using Comparative CT (SDS v 2.0.6 Software)* (Thermo Fisher Scientific, Foster City, CA, USA). Serial 10-fold dilutions of a representative sample were used initially to assess that the efficiency of each gene PCR was 90–110%. Cycle threshold (Ct) was obtained for each sample. A corrected Ct (ΔCt) was calculated by subtracting the Ct from the target gene Glut2 Ct for each sample. Relative differences from the control sample were then calculated by using the formula: fold change 2^Δ^Ct -sample ΔCt (Schmittgen and Livak, 2008). Analysis was carried out as a one-step RT-PCR, the protocol is set by Li et al. (2003) and TaqMan® RNA-to-CT™ 1-Step Kit protocol. The reaction mixture contained: 100 ng RNA, 500 nM forward primers, 500 nM reverse primers, 200 nm TaqMan® probes, TaqMan® RT-PCR Mix (2X) and TaqMan® RT Enzyme Mix (40X) (Thermo Fisher Scientific, Foster City, CA, USA). Primers and probes for cyclophilin (CYC) and glucose transporter 2 (GLUT2) (Table 1.)
Table 1. Primer and probe sequences for real-time RT-PCR

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence 5’-3’</th>
<th>GenBank</th>
</tr>
</thead>
</table>
| Cyc  | F: CCCACCGTGTTCTTCGACAT  
      R: TGCAAACAGCTCGAAGCAGA  
      P: CAAGGGGTCGCCATCAGCCG | M19533 |
| Glut2| F: GTCCAGAAAGCCCCAGATACC  
      R: TGCCCCCTTAGTCTTTTCAAGCT  
      P: TTGCCCTGACTTCCTCTTCCAAATTTAGGTAA | NM 012879 |

The reaction condition was: 48°C for 15 min for RT; one cycle of 95°C for 10 min plus 40 cycles of 95°C for 15 s and 60°C for 1 min for PCR. The mRNA level of GLUT2 gene was expressed as its ratio to internal control gene– cyclophilin (CYC). Data are reported as means ±S.E.M. Fold effects of glucose transporter 2 (GLUT2) expression in STZ treated rats was compared to untreated rats and was determined after normalization with CYC expression.

Statistics
The significance between group differences (mean ± SD) was tested by Mann-Whitney U test, with significance set at p ≤ 0.05.

A  Protein expression

B  mRNA expression
Figure 1. Protein and mRNA glucose transporter 2 expression in hippocampus and parietal and temporal cortices. Animals were euthanized 1 month after intracerebroventricular (icv) treatment with streptozotocin (STZ-icv) (1.5 mg/kg) or vehicle (CTR). Glucose transporter 2 (GLUT2) protein expression in cortices (parietal/PC and temporal/TC) and hippocampus (HPC) were measured by Western blot analysis with the representative blots being presented (A). β-actin was used as a loading control. Each bar (mean±SEM) represents relative ratio of signal intensity presented with arbitrary units (au) for CTR and STZ (A). Relative expression of GLUT2 mRNA in TC, PC, and HPC in CTR and STZ groups was measured by real-time RT-PCR. Expression of GLUT2 was normalized to expression of cyclophilin (CYC) and then fold effects were determined by comparing expression of GLUT2 in TC, PC, and HPC to expression in random TC sample. Each bar represents the mean ±S.E.M. of 4-6 experiments (and each experiment contained three samples that were also averaged) (B). *p<0.05 vs CTR by Mann-Whitney U test for both analysis.

Literature:

Li B, Xi X, Roane DS, Ryan DH, Martin RJ. Distribution of glucokinase, glucose transporter GLUT2, sulfonylurea receptor-1, glucagon-like peptide-1 receptor and neuropeptide Y messenger RNAs in rat brain by quantitative real time RT-PCR. Brain Res Mol Brain Res. 2003;113(1-2):139-42.