Supplemental data.1

Table 1. Stereotactic coordinates for placements of infusion guiding cannula and i.c.v. probes.

(mm)	Anteroposterior	Lateral	Ventral	Angle
i.c.v.	-0.8	2.0	-3.2	0
PVN	-1.6	2.0	-7.9	10

Tooth bar was set as -3.2 mm. The ventral coordinates were standardized for 300g BW, every additional 25g BW will be placed 0.1 mm deeper.

Supplemental data.2

Immunohistochemistry

Animals were deeply anaesthetized after the final blood sample and 1 µl colorful dye was injected via the i.c.v. guiding probe to validate the probe placement. Animals were transcardially perfused with saline, followed by a solution of 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4) at 4℃. Tissues were cryoprotected for 48hrs with 30% sucrose in 0.1M Tris-buffered saline (TBS), the brain tissue was cut into 35 µm coronal sections and divided into five equal groups for single immunohistochemical staining or double-labeling immunofluorescence staining. For single Fos immunohistochemistry, sections were incubated overnight at 4℃ with goat anti-Fos (1:1500; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) primary antibodies. Sections were then rinsed in 0.1M TBS, incubated 1 hr in biotinylated horse anti-goat IgG, and then 1 hr in avidin–biotin complex (ABC, Vector Laboratories, Inc., Burlingame, CA). The reaction product was visualized by incubation in 1% diaminobenzidine (DAB) (0.05% nickel ammonium sulphate was added to the DAB solution to darken the reaction product, DAB/Ni) with 0.01% hydrogen peroxide for 5-7 min. Sections were mounted on gelatin-coated glass slides, dried, run through ethanol and xylene and covered for evaluation by light microscopy. To check whether the Fos-ir was co-localized within arginine vasopressin (AVP) containing neurons in the PVN, adjacent sections were incubated with both goat anti-Fos and rabbit anti-AVP (1:1000, Boehringer Mannheim, Indianapolis, IN) primary antibodies. After Fos staining with DAB/Ni, sections were rinsed and incubated with horse anti-rabbit IgG and ABC, and only DAB staining was applied to visualize the AVP-ir.

Double-labeling immunofluorescence was applied to characterize the relation between the pre-autonomic neurons in the PVN that project to the spinal cord and the PACAP-38-induced Fos-ir. Sections were incubated overnight at 4 ℃ with goat anti-Fos primary antibody, rinsed in 0.1M TBS, incubated 1 hr in biotinylated horse anti-goat IgG, and rinsed and incubated with streptoavidin-Cy2 for 1 hr, rinsed, mounted on gelatin-coated glass slides, dried and covered with glycerol in 0.1 M PBS (PH 9.0) and evaluated using confocal laser scanning microscopy.

Supplemental data.3

Liver mRNA was isolated using a Magna Pure LC mRNA tissue kit (Roche Molecular Biochemicals). cDNA synthesis was subsequently performed using a first strand cDNA synthesis kit for RT-PCR with oligo d(T) primers (Roche Molecular Biochemicals). PCRs were performed on a Lightcycler (Roche Molecular Biochemicals) using SYBR Green (Roche Molecular Biochemicals), 50 ng forward and reversed strand primers (Biolegio, Nijmegen, The Netherlands). Analysis of the PCR data was performed using linear regression.

RT-PCR primer

Gene	Forward primer	Reverse primer
Pepck	TGCCCTCTCCCCTTAAAAAAG	CGCTTCCGAAGGAGATGATCT
G6Pase	CCCATCTGGTTCCACATTCAA	GGCGCTGTCCAAAAAGAATC
HPRT	GCAGTACAGCCCCAAAATGG	AACAAAGTCTGGCCTGTATCCAA

*HPRT: hypoxanthine phosphoribosyltransferase, as reference gene.

Supplemental data.4

For counting the Fos-immunoreactive neurons in the PVN, images were captured by a computerized image analysis system consisting of an Axioskop 9811-Sony XC77 black and white video camera (Sony Corp., Tokyo, Japan). In the images, the whole area of PVN was outlined manually on both sides of the third ventricle. The Fos-positive nuclear profiles were automatically segmented by a dedicated macro written within the ImagePro programming environment. For each rat, four sections were measured 130μm apart (from bregma ≈ -1.4 to -1.9 mm); the mean number of Fos positive nuclear profiles from these four sections was calculated. All values are expressed as the mean ± SEM/per section, and data were analyzed using one-way ANOVA. Statistical significance was set at $P < 0.05$.

Supplemental Figure 1 Control infusion of VPAC1R and VPAC2R agonists into the systemic circulation or i.c.v. infusion of PAC1R, VPAC2R and MC3/4-R antagonists without PACAP-38 had no effects on either plasma glucose concentrations (**A & B**) or endogenous glucose production (EGP) (**C & D**). Data are presented as mean±SEM.