Supplemental data

ANGIOTENSIN II TYPE 1 RECEPTOR SIGNALING REGULATES FEEDING BEHAVIOR THROUGH ANOREXIGENIC CORTICOTROPIN-RELEASING HORMONE IN HYPOTHALAMUS

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EXPERIMENTAL PROCEDURES

Serum corticosterone measurement - Serum corticosterone concentrations were determined using Corticosterone EIA Kit (Enzo Life Sciences, Farmingdale, NY, USA) according to the manufactures' protocol.

Real time RT-PCR analysis - Total RNA was extracted by using RNeasy Kit (Qiagen, Hamburg, Germany), and single-stranded cDNA was transcribed by using QuantiTect Reverse Transcription Kit (Qiagen, Hamburg, Germany), according to the manufacturer's protocol. We conducted quantitative real-time PCR analysis with the Universal ProbeLibrary Assays (Roche Applied Science, Indianapolis, IN, USA), according to the manufacturer's instructions. Amplification conditions were initial denaturation for 10 min at 95°C followed by 45 cycles of 10 s at 95°C and 25 s at 60°C. Individual PCR products were analyzed by melting-point analysis. The expression level of a gene was normalized relative to that of *Gapdh* by using a comparative Ct method. The primer sequences and Universal Probe numbers were designed with the ProbeFinder software as following: *Agtr1a*, 5'-actcacagcaaccctccaag-3' and 5'-ctcagacactgttcaaaatgcac-3', No. 9; *Agtr1b*, 5'-cgccagcagcactgtaga-3' and 5'-ggaggggtgaattcaaaa-3', No. 32; *Agtr2*, 5'-ggagctcggaactgaaagc-3' and 5'-ctgcagcaactccaaattctt-3', No. 41.

High-fat feeding – Mice were allowed free access to water and high-fat chow (D12492; Research Diets, New Brunswick, NJ, USA) from 6 to 12 weeks of age. The daily food intake and body weights were measured.

Blood pressure measurement – The systolic and diastolic blood pressures and pulse rates were measured in conscious mice noninvasively by a programmable sphygmomanometer (BP-98A,

Softron, Tokyo, Japan) using the tail-cuff method.

Peripheral administration of olmesartan – Olmesartan (1.2 mg/kg/day; Daiichi-Sankyo, Tokyo, Japan) or vehicle was administered subcutaneously to 8-week-old wild-type mice using osmotic mini-pumps (ALZET model 2004; Durect, Cupertino, CA, USA). Two weeks after the implantation, the body weights, blood pressures, pulse rates, and daily food intake were analyzed. Three weeks after the implantation, mice were sacrificed and brain tissues were subjected to *in situ* hybridization analysis.

I.c.v. administration of olmesartan – Olmesartan (0.12 mg/kg/day or 1.2 mg/kg/day) or vehicle was administered i.c.v. to 8-week-old wild-type mice using osmotic mini-pumps (ALZET model 1004; Durect, Cupertino, CA, USA), which was attached to an ALZET Brain Infusion Kit 3 (Durect, Cupertino, CA, USA). Mice were anesthetized with ether, and the tip of the infusion catheter was positioned in the third ventricle. The cannula was secured with Loctite 454 (Henkel, Düsseldorf, Germany), and the pump was placed subcutaneously on the dorsal side. Two weeks after the implantation, daily food intake was measured.