Supplementary Materials 2

In situ hybridization

Chronically cannulated rats were divided into two groups (n = 4), anesthetized with sodium pentobarbital (50 mg/kg, i.p.) 1 h after ICV injection with phosphate buffere saline (PBS) or UCN (300 pmol/10 μL). Then they were transcardially perfused with saline followed by tissue fixative (Genostaff Co. Ltd) brains were dissected. Fixed whole brains were cut along the coronary plane, embedded in paraffin, and sectioned at 6 μm for *in situ* hybridization performed according to procedures described previously (Noguchi *et al*, 2008). The cDNA template used for *c-fos* was a 458 base pair fragment corresponding to bases 884–1341 of *fos* cDNA (GenBank accession no. NM_022197.2). Sense and antisense cRNA probes for *c-fos* mRNA were synthesized using a DIG RNA Labeling Kit (Roche, Switzerland) according to the manufacturer's protocol. Each positive cell was counted unilaterallary in PVN, paraventricular nucleus; VMH, ventromedial hypothalamus; LC, locus ceruleus; NTS, nucleus of the solitary tract; DMV, dorsal motor nucleus of the vagus; and RVLM, rostral ventrolateral medulla sections and averaged bilaterally for 1section (n=4/group).

Reference

Noguchi M, Yuzurihara M, Kase Y, Yasui T, Irahara M (2008). Involvement of cytokine-induced neutrophil chemoattractant in hypothalamic thermoregulation of luteinizing hormone-releasing hormone. *Endocrinology* **149**(6): 2899-2906.