Genetics and Molecular Research

Effect of puerarin on the expression of NMU, NPY, and POMC genes in the hypothalamus

Q.D. Jiang[1,2] and X.P. Li[1]

[1]College of Veterinary Medicine, Northwest A&F University, Yangling, Shanxi, China
[2]Key Laboratory of Animal Biochemistry and Nutrition, Ministry of Agriculture, Henan University of Animal Husbandry and Economy, Zhengzhou, Henan, China

Corresponding author: X.P. Li
E-mail: jiangqd2000@126.com

Genet. Mol. Res. 15 (1): gmr.15017362
Received August 4, 2015
Accepted October 26, 2015
Published February 26, 2016
DOI http://dx.doi.org/10.4238/gmr.15017362

ABSTRACT. The hypothalamus is an important component of the nervous system, and neuropeptide Y (NPY), proopiomelanocortin (POMC), and neuromedin U (NMU) are key players in physiological regulation. Puerarin is important for nerve regulation. We investigated the effect of puerarin on the expression of NMU, NPY, and POMC genes in the hypothalamus. The results showed that the puerarin low-dose group and the other groups were significantly different ($P < 0.05$). However, there was no significant difference in NMU, POMC, and NPY among the groups.

Key words: Puerarin; NMU; NPY; POMC; Gene expression

INTRODUCTION

Puerarin [4H-1-benzopyran-4-one,8-D-glueopyranosyl-7-hydroxy-3-(4-hydroxy-phenyl), $C_{21}H_{20}C_9$] is one of 3 major isoflavonoid compounds isolated from *Puerarina lobata*, an edible vine widely used for various purposes. Radix Puerariae (pueraria) is known to be cerebroprotective (Ye et al., 2003). The main components include puerarin, 3'-methoxy-puerarin, daidzin, daidzein, and other isoflavones. Puerarin has been demonstrated to be effective in treating brain ischemia (Hu and Xu, 2006; Xu et al., 2007). 3'-Methoxy-puerarin contains a large amount of quaternary carbon and displays physico-chemical properties resembling those of puerarin (Ye et al., 2003). A preliminary study showed that 3'-methoxy-puerarin can protect the brain and improve hemorheology (Zhang et al., 2009). Neuromedin U (NMU) is a neuropeptide originally isolated from the spinal cord (Minamino et al., 1985). The first biological activity ascribed to NMU was smooth muscle contraction of the uterus, but now it is also known to reduce food intake and body weight (Howard et al., 2000), regulate circadian rhythm, and promote pronociceptive effects (Nakahara et al., 2004; Moriyama et al., 2004). A number of neuropeptides, such as substance P (SP) and neuropeptide Y (NPY) are expressed in central and peripheral nerve cells and are known to directly activate mast cells, triggering inflammation and promoting anaphylactic responses (Suzuki et al., 1999; Naveilhan et al., 2001).

MATERIAL AND METHODS

Hypothalamus isolation

The rats were anesthetized and blood drawn. The following surgical procedure was utilized: along the sagittal suture, the skin was excised; a cut was made on both sides of the parietal, temporal bone, and dura mater; and the brain tissue was removed. Under an anatomic microscope, the hypothalamus was separated and immediately preserved in liquid nitrogen. The hypothalamus weight in each group was close to average.

RNA extraction and reverse transcription

Total RNA was extracted from the hypothalamus using the TRIzol reagent kit (Invitrogen Inc., Minneapolis, MN, USA) and used to determine the NMU, NPY, and proopiomelanocortin (POMC) mRNA levels by reverse transcription polymerase chain reaction (RT-PCR). All extracted RNA samples were finally dissolved in 30 µL RNase-free water. The purity of the dissolved RNA was assessed by the A260/A280-nm ratio that was measured using an ultraviolet/visible spectrophotometer (Nanodrop 2000/2000C, Rocky Hill, SC, USA). The integrity of the RNA was determined by denaturing agarose gel electrophoresis. RNA was reverse transcribed with a reaction mix containing 8.5 µL diethylpyrocarbonate-H_2O, 3 µL 5X buffer, 3 µL 2.5 mM dNTP mix, 1 µL 50 pM Oligo (dT)18, 1 µL 5 U/µL AMV, 2.5 µL 40 U/µL RNase inhibitor, and 1 µL RNA. The total reaction volume was 20 µL. After gently mixing, the solution was incubated for 60 min at 42°C and 15 min at 72°C and 2 min in an ice bath. Two controls were performed: one control was prepared using all reagents except the RNA sample, for which an equivalent volume of water was substituted, and the other control was prepared using all reagents except reverse transcriptase. The controls underwent identical PCR procedures as the experiment samples. The cDNA product was stored at -20°C.

RT-PCR

The primers used for amplification of NMU, NPY, and POMC mRNA were designed using the Primer Premier TM Version 5.0 software (PREMIER Biosoft International, Victoria, Canada) and checked by basic local alignment tool searches. All the primers, including those for the β-actin gene that was used as an internal reference, were synthesized by Shanghai Sango Biological Engineering Technology & Services Co. Ltd. (China). Electrophoresis on 1.2% (w/v) agarose gels was conducted to determine the quality and integrity of the primers. The sequence of primers and product size were as follows: NMU, sense 5'-atctcctctcctgtcactgc-3', antisense 5'-gttgacctcttcccattgcgt-3', product 450 bp (NM-022239); NPY, sense 5'-gctgtgtgactgaccctgg-3', antisense 5'-gggacaggcagactgtttc-3', product 354 bp (NM-012614); POMC, sense 5'-gaacagcccttgactgaaaa-3', antisense 5'-ctgagagcg tcacag-3', product 471 bp (AH-002232); β-actin, sense 5'-cctctatgccaacacagtgc-3', antisense 5'-atactcctgcttgctgatcc-3', product 207 bp. The cDNAs were further amplified by PCR in a 25-μL mix consisting of 1 μL RT reaction solution, 12.5 μL 2X master mix, 1 μL 20 pM forward primer, 1 μL 20 pM reverse primer, and 9.5 μL sterilized H_2O. The reaction substrates were mixed by gently flicking the bottom of each tube. PCR amplification was carried out for 35 cycles (94°C, 15 s; 57°C, 15 s; 72°C, 30 s) for β-actin, 35 cycles (94°C, 10 s; 55°C, 15 s; 72°C, 35 s) for NMU, 35 cycles (95°C, 10 s; 58°C 20 s; 72°C 30 s) for NPY and 35 cycles (94°C, 15 s; 56°C, 15 s; 72°C 30 s) for POMC. Reactions were completed with a final extension at 72°C for 10 min. For each 5-μL sample, the PCR amplification products were mixed gently with 4 μL of fluorochrome before protection from light for 10 min, and then they were visualized on a 1.2% agarose gel with a multi-image light system.

Statistical treatment of results

All results are reported as means ± SD and were analyzed using the Statistical Package for the Social Sciences statistical software (version 17.0). Differences between group data were analyzed using the Student-Newman-Keuls post hoc test of one-way analysis of variance, and differences between groups were evaluated using the paired-samples <0.01 in 2-tailed tests.

RESULTS

The integrity of the total RNA that was extracted from hypothalamus and assessed by gel electrophoresis is shown in Figure 1. The amplified NMU, NPY, and POMC genes are shown in Figure 2. The relative expression levels of NMU, NPY, and POMC mRNA in the hypothalamus from the 6 different groups are shown in Figure 3.

The puerarin high-dose group was significantly increased compared with the model group ($P < 0.05$) while the puerarin low-dose group and the other groups were significantly different ($P < 0.05$). It was suggested that puerarin induced NMU up-regulation. Puerarin induced NPY down-regulation compared to the normal group. Puerarin induced POMC up-regulation. NMU was also increased. However, there was no significant difference in NMU, POMC, and NPY expression among the groups. The results show that the action of puerarin may be related to NPY and POMC expression.

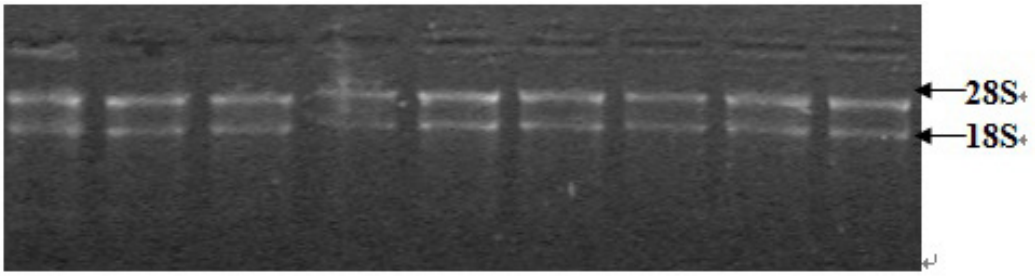

Figure 1. Total RNA that was extracted from hypothalamus.

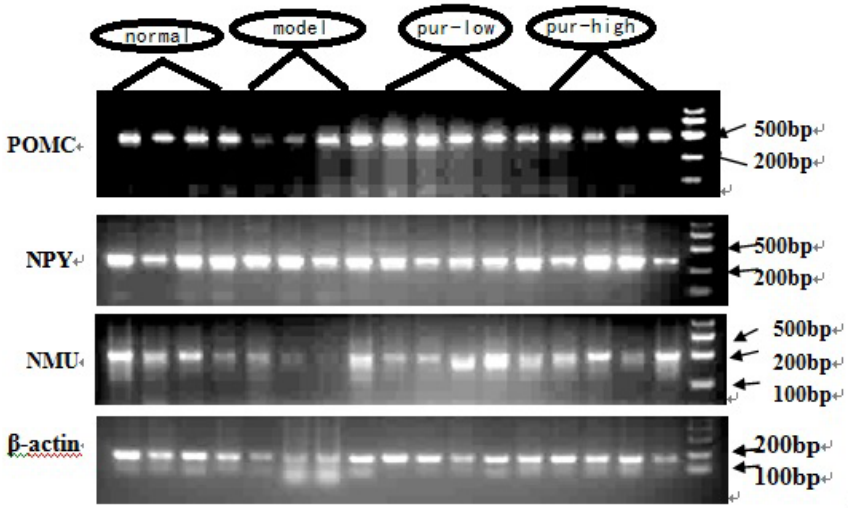

Figure 2. POMC, NPY, and NMU amplified genes.

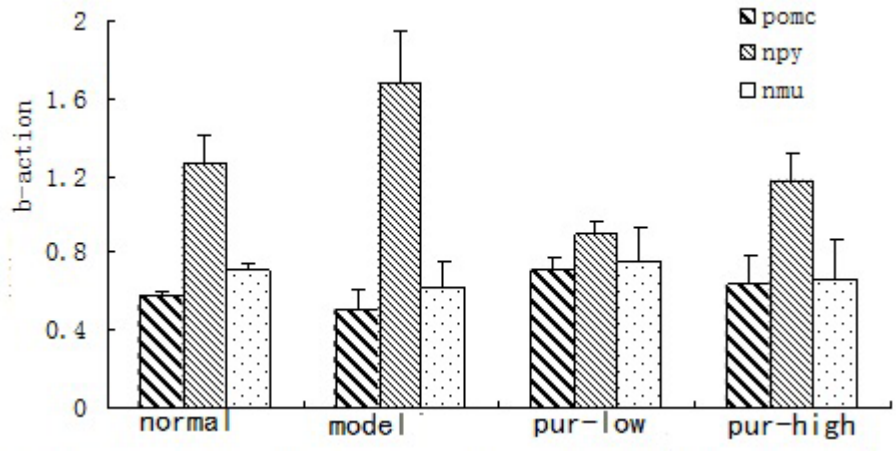

Figure 3. Effect of puerarin to the expression of POMC, NPY and NMU genes in hypothalamus.

DISCUSSION

NMU mobilizes intracellular Ca^{2+} stores in NMU-R1- or NMU-R2-expressing cells, suggesting that these receptors couple to members of the Gq11 subfamily of G proteins (Brighton et al., 2004). While NMU-R2 is localized specifically in nerve cells, NMU-R1 is abundantly expressed in various types of immune cells including NK cells, T cells, and monocytes (Hedrick et al., 2000). The ligand NMU itself has been shown to be expressed in APCs, including dendritic cells, monocytes, and B cells (Hedrick et al., 2000). Furthermore, NMU-R1 has been shown to be expressed in a mouse Th2 clone, and NMU promotes the secretion of various cytokines (Johnson et al., 2004). NMU-R1 is also highly expressed in mast cells, and NMU induces mast cell degranulation, which leads to early-phase inflammation such as vasodilation, extravasation, and neutrophil infiltration in inflamed sites (Moriyama et al., 2005). Moreover, SP, NPY, calcitonin gene-related peptide, and somatostatin are also known to regulate T cell adhesion to fibronectin via the activation of b1-integrin (Levite et al., 1998). In macrophages, these neuropeptides also regulate cytokine production under inflammatory conditions (Kincy-Cain and Bost, 1997; Delgado et al., 1999). Neuropeptide-Y/agouti-related peptide inter neurons and proopiom elanocortin POMC/ cocaine- and amphetamine-regulated transcript neurons play an important role in leptin regulation of the hypothalamic-pituitary axis (Mercer et al., 2000; Bouret et al., 2001; Morrison et al., 2005). However, little is known about how leptin regulates reproductive functions through POMC neurons. *In vitro* studies have demonstrated that puerarin induced NMU and POMC up-regulation and NPY down-regulation. Puerarin may therefore affect NPY and POMC.

ACKNOWLEDGMENTS

Research supported by the Project of National Major Basic Dairy Research “973” Plan (#2011CB100802) and by the grant of Ministry of Agriculture (#2011-G35).

REFERENCES

Bouret S, Chuoi-Mariot MT, Prevot V, Croix D, et al. (2001). Evidence that TGF beta may directly modulate POMC mRNA expression in the female rat arcuate nucleus. *Endocrinology* 142: 4055-4065.

Brighton PJ, Szekeres PG, Wise A and Willars GB (2004). Signaling and ligand binding by recombinant neuromedin U receptors: evidence for dual coupling to Galphaq/11 and Galphai and an irreversible ligand-receptor interaction. *Mol. Pharmacol.* 66: 1544-1556. http://dx.doi.org/10.1124/mol.104.002337

Delgado M, Sun W, Leceta J and Ganea D (1999). VIP and PACAP differentially regulate the costimulatory activity of resting and activated macrophages through the modulation of B7.1 and B7.2 expression. *J. Immunol.* 163: 4213-4223.

Hedrick JA, Morse K, Shan L, Qiao X, et al. (2000). Identification of a human gastrointestinal tract and immune system receptor for the peptide neuromedin U. *Mol. Pharmacol.* 58: 870-875.

Howard AD, Wang R, Pong SS, Mellin TN, et al. (2000). Identification of receptors for neuromedin U and its role in feeding. *Nature* 406: 70-74. http://dx.doi.org/10.1038/35017610

Hu J and Xu DH (2006). Recent research progress of pharmacology and clinical for Puerarin. *Shijie Linchuang Yaowu* 27: 215-220.

Johnson EN, Appelbaum ER, Carpenter DC, Cox RF, et al. (2004). Neuromedin U elicits cytokine release in murine Th2-type T cell clone D10.G4.1. *J. Immunol.* 173: 7230-7238. http://dx.doi.org/10.4049/jimmunol.173.12.7230

Kincy-Cain T and Bost KL (1997). Substance P-induced IL-12 production by murine macrophages. *J. Immunol.* 158: 2334-2339.

Levite M, Cahalon L, Hershkoviz R, Steinman L, et al. (1998). Neuropeptides, via specific receptors, regulate T cell adhesion to fibronectin. *J. Immunol.* 160: 993-1000.

Mercer JG, Moar KM, Ross AW, Hoggard N, et al. (2000). Photoperiod regulates arcuate nucleus POMC, AGRP, and leptin receptor mRNA in Siberian hamster hypothalamus. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 278: R271-R281.

Minamino N, Kangawa K and Matsuo H (1985). Neuromedin U-8 and U-25: novel uterus stimulating and hypertensive peptides identified in porcine spinal cord. *Biochem. Biophys. Res. Commun.* 130: 1078-1085. http://dx.doi.org/10.1016/0006-291X(85)91726-7

Moriyama M, Furue H, Katafuchi T, Teranishi H, et al. (2004). Presynaptic modulation by neuromedin U of sensory synaptic transmission in rat spinal dorsal horn neurones. *J. Physiol.* 559: 707-713. http://dx.doi.org/10.1113/jphysiol.2004.070110

Moriyama M, Sato T, Inoue H, Fukuyama S, et al. (2005). The neuropeptide neuromedin U promotes inflammation by direct activation of mast cells. *J. Exp. Med.* 202: 217-224. http://dx.doi.org/10.1084/jem.20050248

Morrison CD, Morton GJ, Niswender KD, Gelling RW, et al. (2005). Leptin inhibits hypothalamic Npy and Agrp gene expression via a mechanism that requires phosphatidylinositol 3-OH-kinase signaling. *Am. J. Physiol. Endocrinol. Metab.* 289: E1051-E1057. http://dx.doi.org/10.1152/ajpendo.00094.2005

Nakahara K, Kojima M, Hanada R, Egi Y, et al. (2004). Neuromedin U is involved in nociceptive reflexes and adaptation to environmental stimuli in mice. *Biochem. Biophys. Res. Commun.* 323: 615-620. http://dx.doi.org/10.1016/j.bbrc.2004.08.136

Naveilhan P, Hassani H, Lucas G, Blakeman KH, et al. (2001). Reduced antinociception and plasma extravasation in mice lacking a neuropeptide Y receptor. *Nature* 409: 513-517. http://dx.doi.org/10.1038/35054063

Suzuki R, Furuno T, McKay DM, Wolvers D, et al. (1999). Direct neurite-mast cell communication *in vitro* occurs via the neuropeptide substance P. *J. Immunol.* 163: 2410-2415.

Xu XH, Zheng XX, Zhou Q and Li H (2007). Inhibition of excitatory amino acid efflux contributes to protective effects of puerarin against cerebral ischemia in rats. *Biomed. Environ. Sci.* 20: 336-342.

Ye JL, Zhou XW and Chen Z (2003). 2D NMR study on solution structure of natura1 product 3-methoxy puerarin. *Xiamen Daxue Xuebao.* 42: 69-72.

Zhang YB, Du GY, Xiong YL. (2009). The effects of 3'-methoxy-puerarin on thrombus formation and hemorheology in rats. *Zhongyao Yaoli yu Linchuang*. 25: 29-31.