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Peptides 26 (2005) 675–681
PEPTIDES
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# Characterization of a naturally-occurring polymorphism in the UHR-1 gene encoding the putative rat prolactin-releasing peptide receptor

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Received 20 September 2004; received in revised form 23 November 2004; accepted 24 November 2004
Available online 12 January 2005

**Abstract**

The rat orphan receptor UHR-1 and its human orthologue, GPR10, were first isolated in 1995. The ligand for this receptor, prolactin-releasing peptide (PrRP), was identified in 1998 by reverse pharmacology and has subsequently been implicated in a number of physiological processes. As supported by its localization and regulation in the hypothalamus and brainstem, we have shown previously that PrRP is involved in energy homeostasis. Here we describe a naturally occurring polymorphism in the UHR-1 gene that results in an ATG to ATA change at the putative translational initiation site. The presence of the polymorphism abolished the binding of $^{125}$I PrRP in rat brain slices but did not affect the ability of PrRP to reduce fast-induced food intake. Together this data suggest that PrRP may be exerting its feeding effects through a receptor other than UHR-1.


*Keywords:* UHR-1; PrRP; Food intake; Receptor autoradiography; Genetic variation

## 1. Introduction

The rat orphan receptor UHR-1 was first identified in 1995 following PCR amplification using degenerate primers from the hypothalamic suprachiasmatic nucleus [36]. This was rapidly followed by the identification from genomic DNA, of the human form of the receptor, GPR10 [19]. The UHR-1/GPR10 receptor displays 31% amino acid identity with the neuropeptide Y1 (NPY-1) receptor, but also shares sequence identity with receptors for tachykinins (substance P and substance K), somtostatin (SSTR3 and SSTR5) and opioids (delta, mu and kappa) [19,36]. In the rat brain, UHR-1 mRNA is found in the reticular thalamic nucleus (RT), the paraventricular nucleus (PVN), the periventricular nucleus (PeV), dorsal medial hypothalamus (DMH), area postrema (AP) and nucleus of the tractus solitarius (NTS) [7,25]. UHR-1 mRNA is also expressed in the anterior pituitary, adrenal medulla, testis and epididymis [7,23].

The ligand for the UHR-1/GRP10 receptor, named prolactin-releasing peptide (PrRP) due to its ability to induce prolactin release from cultured pituitary cells, was first isolated by reverse pharmacology in 1998 following screening of bovine hypothalamic extracts [9]. PrRP immunoreactivity has a very discrete localization, and is only found in the DMH, NTS and ventrolateral medulla (VLM) [3,20,37]. Receptor autoradiography studies indicate that $^{125}$I $PrRP_{1–31}$ displays high levels of binding in the RT, and lower levels of binding in the PeV/PVN. No binding was described in the other areas where UHR-1 is expressed [25]. Since the original paper describing PrRP as a novel prolactin-releasing factor [9], there have been a number of studies that have questioned its role in prolactin release in vivo and in vitro [12,27,33,35]. Other proposed functions of PrRP include cardiovascular [26], pain

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doi:10.1016/j.peptides.2004.11.020

[13] and sleep regulation [38], mediation of stress responses [21], oxytocin [39], leutenising hormone (LH) and follicle-stimulating hormone (FSH) release [10,31,34], growth hormone secretion [11], and the regulation of food intake [16,30].

We have demonstrated that centrally administered PrRP reduces both nocturnal and fast-induced food intake and body-weight gain [16], and this finding was independently confirmed by Seal et al. [30]. PrRP reduces food intake without disrupting the normal behavioral satiety sequence or through causing a taste aversion [17], thus we have developed a hypothesis relating PrRP to the physiological regulation of appetite and energy balance [4,5,18]. This hypothesis was given additional credence by the discovery that a mouse deficient in GPR10 is overweight [8]. To date, attempts to relate polymorphisms in the human GPR10 gene to obesity have failed [1]. In this paper we describe a naturally occurring polymorphism in the start codon of the UHR-1 gene in rats, and how this mutation affects $^{125}$I PrRP binding and the anorectic effect of PrRP.

## 2. Materials and methods

### 2.1. Animals and materials

Unless otherwise stated, all chemicals were obtained from Sigma-Aldrich (Poole, UK). All experiments were performed using adult male rats (250–300 g) of the following strains: Sprague–Dawley (Charles River, Margate, UK) and Hooded Lister (Harlan UK, Bicester, UK). Animals were kept in a 12 h light:12 h dark cycle (lights on, 08:00 h–20:00 h) at 21 ± 1 °C and 45 ± 10% humidity with ad libitum access to food and water. All experiments were performed in accordance with the UK Animals (Scientific Procedures) Act 1986.

### 2.2. Amplification of UHR-1 and polymorphism screen of PCR products

Rats ($n = 3$ per strain) were killed by exposure to a rising concentration of carbon dioxide followed by cervical dislocation and a sample of liver removed. Tissue was homogenized in a pestle and mortar, and DNA extracted using the method of Laird et al. [15]. PCR was performed in a 50 μl volume containing 1 U Amplitaq Gold™ (Perkin-Elmer, NJ, USA), 100 μM dNTPs, 10 pmol of each primer (segment 1: 5′-NNNAAGCTTGTTCACAGGTGG-3′ and 5′-AGGAAGAAAACAAGGTGGCACA-3′; segment 2: 5′-CTTGTCCGATGTGCTCATGTG-3′ and 5′-AGTCTAGAGGGCTGGGTAGGA-3′) and 2 μl genomic DNA in a Hybaid PCR Express Thermocyler (Hybaid Ltd., UK). Cycling conditions were: 10 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 58 °C and 1 min at 72 °C. A final extension step of 72 °C for 10 min was performed. A no template control was included in the amplification of each segment. PCR products were separated on a 2% agarose ethidium-bromide gel. PCR products were sequenced directly using ET Dye terminator chemistry (performed according to the manufacturers instructions; Amersham Pharmacia, Little Chalfont, UK) and a MegaBASE capillary sequencer (Molecular Dynamics, Sunnyvale, USA). Sequencing data revealed a guanidine to adenine nucleotide difference at position 23 of the published UHR-1 sequence (accession number S77867), such that the gene start codon changed from ATG to ATA.

### 2.3. Engineered restriction fragment length polymorphism (eRFLP)

Genomic DNA was extracted from liver samples using the method of Laird et al. [15]. PCR was used to generate a 280-bp product, engineered to include a BspH1 restriction recognition site at the site of the polymorphism. PCR was performed in a 50 μl volume containing 45 μl Reddy Taq™ (2.0 mM $MgCl_2$; AB gene, Epsom, UK), 10 pmol of forward and reverse primer (5′-CAATTTGATTACCTTTGGACAGGTGTCAT-3′, 5′-CAGCACAAGAAGGCAGTTGCCCACCAGACC-3′) and 5% DMSO. PCR cycling conditions were as follows; 10 min at 95 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 58 °C and 1 min at 72 °C. A final extension step of 72 °C for 10 min was performed. The products were run on a 2% agarose ethidium bromide gel to check their size. The PCR products were cut using BspH1 to give a 250-bp product; 10 μl PCR product was incubated at 37 °C for 4 h with 5 U BspH1 (New England Biolabs, Hitchin, UK), 2 μl 10× NE Buffer 4 and made up to 20 μl with nuclease free water. The resulting digest was run on a 2% agarose ethidium bromide gel to separate the products. Cleavage by the restriction enzyme BspH1 only occurred if the animal did not contain the ATG to ATA nucleotide change in the start codon.

### 2.4. Effect of PrRP on fast-induced feeding

Animals had lateral ventricular cannulae inserted (0.8 mm posterior and 1.5 mm lateral to bregma and 3.0 mm below dura [24]) under 2.5% halothane (AstraZeneca, Macclesfield, UK) anesthesia. The animals were allowed to recover and experiments were performed 1 week later. They were housed individually at 10:00 h without food, but with free access to water, and left to acclimatize. At 10:00 h the following day (after a 24-h fast) the conscious, unrestrained animals were injected with 4 nmol rat $PrRP_{1–31}$ in 2 μl 0.9% sterile saline (i.c.v.; Peptide Inc., Osaka, Japan) or 2 μl 0.9% sterile saline (vehicle). Food was returned to the animals immediately after injection, and food intake was measured 1 h later. Cannulae placement for each animal was verified after the experiment by monitoring the drinking response to i.c.v. administration of 50 ng angiotensin II in 2 μl sterile saline. Animals that failed to drink a minimum of 5 ml water in the 10 min post-injection were excluded from the data analysis. Where applicable, after completion of the experiment, animals were