Neuroscience 156 (2008) 319–333

LEUCINE-RICH REPEAT-CONTAINING G-PROTEIN-COUPLED RECEPTOR 8 IN THE RAT BRAIN: ENRICHMENT IN THALAMIC NEURONS AND THEIR EFFERENT PROJECTIONS

K. SEDAGHAT,[a,b] P.-J. SHEN,[a] D. I. FINKELSTEIN,[b,d] J. M. HENDERSON[e] AND A. L. GUNDLACH[a,c]*

[a]*Howard Florey Institute, The University of Melbourne, Victoria 3010, Australia*

[b]*Centre for Neuroscience, The University of Melbourne, Victoria 3010, Australia*

[c]*Department of Anatomy and Cell Biology, The University of Melbourne, Victoria 3010, Australia*

[d]*Mental Health Research Institute of Victoria, Parkville 3052, Australia*

[e]*Department of Pharmacology, The University of Sydney, New South Wales 2006, Australia*

Abstract—Leucine-rich repeat-containing G-protein-coupled receptor 8 (LGR8; also classified as relaxin family peptide 2 receptor; RXFP2) has been identified as a cognate receptor for the peptide hormone, insulin-like peptide 3 (INSL3) and INSL3-LGR8 signaling plays an essential role in testis descent and germ cell development in human and rodents. *Lgr8* mRNA has been detected in human tissues including testis, kidney and brain, but its regional and cellular distribution in these tissues in human or other species is largely unknown. In an initial step to elucidate the physiological function of a putative INSL3-LGR8 system in rat brain, the localization of *Lgr8* mRNA was investigated using *in situ* hybridization histochemistry, revealing a discrete distribution in forebrain, with expression highly enriched in the thalamus. High densities were detected in the parafascicular nucleus (Pf), the dorsolateral, ventrolateral and posterior thalamic nuclei, and in the medial habenula. *Lgr8* transcripts were also detected in frontal and motor cortices. The comparative distribution of LGR8 (receptor protein) was examined by autoradiography of [^{125}I]-human INSL3 binding sites, with high densities detected in the thalamus, especially in Pf, and in the entire striatum—the caudate putamen (CPμ), islands of Calleja, olfactory tubercle, nucleus accumbens—with lower levels in distinct layers of cerebral cortex. Notably, these areas also receive dopaminergic projections. These findings demonstrate the existence of LGR8 in neuronal soma in the thalamus and axons/terminals in thalamic target areas such as the striatum and frontal cortex. LGR8 was also detected throughout the medial habenula–fasciculus retroflexus–interpeduncular nucleus pathway, further indicating that the receptor is transported from mRNA-expressing soma to remote axonal/terminal sites. These findings suggest the existence of a broadly distributed LGR8 signaling system in the rat involved in sensorimotor, limbic and cognitive functions. Further studies are now required to elucidate the precise function of LGR8, under normal and pathological conditions, as importantly, several of the equivalent receptor-positive areas in human brain are part of the pathology of neurodegenerative conditions including Parkinson's disease. **© 2008 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: insulin-like peptide 3, LGR8/RXFP2, thalamus and basal ganglia, glutamate-dopamine interactions, arousal systems, Parkinson's disease.

Leucine-rich repeat-containing G-protein-coupled receptor 8 (LGR8) is a member of the leucine-rich repeat-containing G-protein-coupled receptor (LGR) family that exert their cellular effects through G-protein activation of secondary messenger cascades (Hsu et al., 2000). Eight LGR genes have been identified and LGRs are classified according to their structural homology: group 1 (LGR1-3) comprises receptors for luteinizing hormone (LH), follicle-stimulating hormone (FSH) and thyroid-stimulating hormone (TSH); group 2 consists of the 'orphan' LGR4, LGR5 and LGR6; and group 3 contains the receptor for relaxin, LGR7, *and* LGR8 (Hsu et al., 2000, 2002). LGR8, also classified as relaxin family peptide 2 receptor; RXFP2 (Bathgate et al., 2006) possesses a high level of amino acid sequence similarity to LGR7 (RXFP1) and both receptors contain an LDL domain at the extracellular N-terminus, followed by 10 leucine-rich repeats and a hinge-like region (Hsu et al., 2002; Hsu, 2003; Scott et al., 2004, 2007). Considerable experimental data have demonstrated that insulin-like peptide 3 (INSL3) which is also known as Leydig insulin-like peptide and relaxin-like factor and belongs to the insulin/relaxin superfamily in mammals (Ivell, 1997; Bathgate et al., 2003; Hsu, 2003), is the native ligand for LGR8.

INSL3 binds with high affinity to recombinant LGR8 transfected into human fetal kidney fibroblast 293T cells, resulting in increased cAMP production (Kumagai et al., 2002; Halls et al., 2005). In addition, mice homozygous for a deletion of the *Lgr8* gene or '*Great*' receptor exhibit cryptorchidism (Overbeek et al., 2001), a common developmental disorder in humans caused by abnormal gubernaculum development that results in defects in spermatogenesis, infertility, and an increased risk of testicular malignancy (Hutson et al., 1997). The phenotype of male *Lgr8*-deficient mice is identical to that of male *Insl3* gene-deficient mice (i.e. bilateral cryptorchidism, defects in testicular descent;

*Correspondence to: A. L. Gundlach, Howard Florey Institute, The University of Melbourne, Victoria 3010, Australia. Tel: +61-3-8344-7324; fax: +61-3-9348-1707.
E-mail address: andrew.gundlach@florey.edu.au (A. L. Gundlach).

Abbreviations: AON, anterior olfactory nucleus; BLA, basolateral amygdaloid nucleus; CM-Pf, centromedian–parafascicular complex; CPu, caudate putamen; ICj, islands of Calleja; INSL3, insulin-like peptide 3; IPAC, interstitial nucleus of posterior limb of anterior commissure; IPN, interpeduncular nucleus; LGR, leucine-rich repeat-containing G-protein-coupled receptor; LGR7/8, leucine-rich repeat-containing G-protein-coupled receptor-7/8 (also classified as RXFP1/2); LOT, nucleus of the lateral olfactory tract; mHb, medial habenular nucleus; M1/2, primary/secondary motor cortex; Pf, parafascicular nucleus.

0306-4522/08 © 2008 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2008.07.029

Nef and Parada, 1999; Zimmermann et al., 1999). In contrast, transgenic overexpression of *Insl3* (and INSL3) in female mice causes the ovaries to descend into the inguinal region due to an over-developed gubernaculum (Adham et al., 2002). Additional studies by Agoulnik and others have confirmed the native INSL3-LGR8 pairing (Bogatcheva et al., 2003; Kamat et al., 2004).

Expression of *LGR8* mRNA has been demonstrated by Northern blot analysis in human testis and gubernaculum and treatment of cultured gubernacular cells with INSL3 stimulated cAMP production and thymidine incorporation (Kumagai et al., 2002). RT-PCR analysis revealed *LGR8* mRNA in a number of human tissues including, testis, uterus, thyroid gland, bone marrow, muscle, kidney and brain (Hsu et al., 2002). In contrast to this broad receptor expression pattern, INSL3 is highly expressed by Leydig cells in the testis (Ivell et al., 1997) and thecal and luteal cells in the ovary (Bathgate et al., 1996), but has not been reported to be highly expressed in other tissues. Although the functional role of INSL3 is established in the developing reproductive system and it has been shown to act in a local autocrine/paracrine fashion in the testis (Kawamura et al., 2004; Feng et al., 2007) and to have a putative role in the kidney (Fu et al., 2006), details of its distribution and function in other organs is unknown.

Similarly, the expression and distribution of LGR8, and the functional role of INSL3-LGR8 signaling in the CNS of humans or experimental animals such as the rat have not been investigated, so the initial aim of the current studies was to explore the regional and cellular distribution of *Lgr8* mRNA in the adult rat brain by *in situ* hybridization histochemistry of multiple [^{35}S]-labeled oligonucleotides. The distribution of functional LGR8 was also investigated by autoradiography of specific [^{125}I]-human INSL3 binding sites. The findings from these studies indicate the enrichment of functional LGR8 in thalamic neurons and their efferent projections and suggest a role for LGR8 in sensorimotor control, via effects on basal ganglia and thalamic, limbic and cortical areas. Preliminary accounts of some of these findings appeared earlier in a monograph on the proceedings of the Relaxin 2004 Conference (Shen et al., 2005) and in communications to learned societies (Sedaghat et al., 2005, 2006).

EXPERIMENTAL PROCEDURES

Animals

All procedures were approved by the Howard Florey Institute Animal Welfare Committee and were performed in strict accordance with guidelines of the National Health and Medical Research Council of Australia. All efforts were made to minimize the number of animals used in this study. For anatomical mapping studies, male Sprague–Dawley rats weighing 250–300 g (Animal Resource Centre, Perth, WA, Australia) were housed two to four per cage with a 12-h light/dark cycle and standard rat chow and water available *ad libitum* until use.

Tissue preparation

Rats were deeply anesthetized and killed by decapitation. Brains were removed, frozen over liquid N_2 and stored at −80 °C until use. For *in situ* hybridization and radioligand binding experiments, coronal sections (14 μm) throughout the rostrocaudal extent of the brain were cut at −16 °C on a cryostat (Reichert Jung 1800; Grale Scientific, Notting Hill, VIC, Australia) and thaw-mounted on microscope slides coated with 0.2% poly-L-lysine (Wisden and Morris, 2002). Sections were air dried for 1 h and stored at −80 °C until use.

In situ hybridization histochemistry

These experiments were carried out using established protocols (Wisden and Morris, 2002; Burazin et al., 2005) with some modifications. For the detection of *Lgr8* mRNA, multiple 39-mer oligonucleotides were designed complementary to different regions of the rat *Lgr8* mRNA sequence. Eight oligonucleotides complementary to nucleotides 55–93, 175–213, 522–560, 1353–1391, 1455–1493, 1601–1639, 1811–1849 and 2000–2038 of the *Lgr8* cDNA sequence (accession number NM001012475) were designed and produced commercially (Sigma-Aldrich, Sydney, NSW, Australia). All cDNA sequences were obtained from the Nucleotide Database of the National Centre for Biotechnology Information (NCBI) (www.ncbi.org). Each oligonucleotide sequence was checked using the database for 100% homology to the target gene, and less than 70% homology with other mammalian genes.

Oligonucleotides (in mixtures of four) were 3′-labeled with [α^{35}S]-dATP (Amersham Biosciences, Amersham, UK) in the presence of terminal deoxynucleotidyl transferase (Roche Diagnostics, Sydney, NSW, Australia) in a $CoCl_2$-buffer to a specific activity of $\geq$1–2$\times 10^9$ dpm/μg. All isotopically-labeled probes (1–5 pg/μl per probe) were diluted in a 'minimal' hybridization buffer containing 50% (v/v) formamide, 10% (v/v) dextran sulfate, 4× SSC (1× SSC, 0.15 M NaCl, 15 mM sodium citrate, pH 7.0) (Wisden and Morris, 2002), and dithiothreitol (200 mM) to minimize disulfide bridge formation and non-specific 'hybridization.' Prior to hybridization, sections were delipidated by incubation in 70–100% ethanol, chloroform and 100% ethanol for 2, 5, 15 min, respectively and then were either dried for hybridization or stored under 100% ethanol in a cold room (4 °C).

Slide-mounted sections were incubated with hybridization buffer at 42 °C overnight and were then washed in 1× SSC at 55 °C for 60 min, rinsed in 1× SSC and dehydrated in ethanol, and exposed for 7–10 days to Kodak Biomax MR film (Integrated Sciences, Sydney, NSW, Australia). Some slides were subsequently dipped in Ilford K5 nuclear emulsion (Ilford Imaging, Melbourne, VIC, Australia), diluted 1:1 with dH_2O, allowed to dry and then exposed in the dark at 4 °C for ~50 days prior to development. Following exposure and development, sections were counterstained with 0.01% Thionin and coverslipped for analysis using bright- and dark-field microscopy.

The specificity of the [^{35}S]-hybridization signal observed in these studies was routinely assessed in a 1:5 series of sections, by the addition of a 100-fold excess of unlabeled oligonucleotides to the hybridization buffer (Fu et al., 2006). In all experiments, hybridization signal that was successfully blocked under these conditions was considered 'specific,' whereas general tissue binding was defined as 'non-specific hybridization.'

[^{125}I]-Human INSL3 binding and *in vitro* autoradiography

Human INSL3 (kindly provided by Prof. John Wade, Howard Florey Institute) was labeled with [^{125}I] to a specific activity of 2000 Ci/mmol by incubation with chloramine-T reagent and purified by HPLC (kindly supplied by Dr. Y.-P. Wan, Perkin-Elmer Life Sciences, Boston, MA, USA and Prof. P. De Meyts, Hagedorn Research Institute, Gentofte, Denmark). These experiments were conducted using established protocols (Burazin et al., 2005), with some modifications. Briefly, to determine the localization of LGR8 binding sites in rat brain, slide-mounted sections were first incubated for 30 min in the 'pre-incubation' buffer (25 mM Hepes,

Download English Version:

https://daneshyari.com/en/article/4340441

Download Persian Version:

https://daneshyari.com/article/4340441

Daneshyari.com