Journal of Psychiatric Research 47 (2013) 1597–1607

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/psychires

Glucocorticoid receptor gene expression and promoter CpG modifications throughout the human brain

Lei Cao-Lei [a,b], Songkiet Suwansirikul [c], Prapan Jutavijittum [c], Sophie B. Mériaux [a], Jonathan D. Turner [a], Claude P. Muller [a,b,*]

[a] Institute of Immunology, Centre de Recherche Public de la Santé, Laboratoire National de Santé, 20A rue Auguste Lumière, L-1950 Luxembourg, Luxembourg
[b] Department of Immunology, Research Institute of Psychobiology, University of Trier, D-54290 Trier, Germany
[c] Faculty of Medicine, Chiang Mai University, Department of Pathology, 50200 Chiang Mai, Thailand

ARTICLE INFO

Article history:
Received 15 February 2013
Received in revised form
5 July 2013
Accepted 22 July 2013

Keywords:
Glucocorticoid receptors
Mineralocorticoid receptors
GR first exon transcripts
GR 3′ splice variants
Methylation status
Human brain

ABSTRACT

Glucocorticoids and the glucocorticoid (GR) and mineralocorticoid (MR) receptors have been implicated in many processes, particularly in negative feedback regulation of the hypothalamic-pituitary-adrenal axis. Epigenetically programmed *GR* alternative promoter usage underlies transcriptional control of GR levels, generation of *GR* 3′ splice variants, and the overall GC response in the brain. No detailed analysis of GR first exons or GR transcript variants throughout the human brain has been reported. Therefore we investigated *post mortem* tissues from 28 brain regions of 5 individuals. *GR* first exons were expressed throughout the healthy human brain with no region-specific usage patterns. First exon levels were highly inter-correlated suggesting that they are co-regulated. *GR* 3′ splice variants (GRα and GR-P) were equally distributed in all regions, and GRβ expression was always low. GR/MR ratios showed significant differences between the 28 tissues with the highest ratio in the pituitary gland. Modification levels of individual CpG dinucleotides, including 5-mC and 5-hmC, in promoters 1D, 1E, 1F, and 1H were low, and diffusely clustered; despite significant heterogeneity between the donors. In agreement with this clustering, sum modification levels rather than individual CpG modifications correlated with GR expression. Two-way ANOVA showed that this sum modification was both promoter and brain region specific, but that there was however no promoter*tissue interaction. The heterogeneity between donors may however hide such an interaction. In both promoters 1F and 1H modification levels correlated with GRα expression suggesting that 5-mC and 5-hmC play an important role in fine tuning GR expression levels throughout the brain.

1. Introduction

The hypothalamus-pituitary-adrenocortical (HPA) axis, one of the major stress response systems, maintains homeostasis and adaptation during challenges (de Kloet et al., 2005). Glucocorticoids (GCs) such as cortisol downregulate the HPA axis activity in a negative feedback loop at the level of the paraventricular nucleus, the pituitary gland and the hippocampus via its cognate receptors: the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). Both the level of, and the balance between the two receptors determine how the tissue responds to GCs. MR has a 10-fold higher affinity for GC than the GR (Reul and de Kloet, 1985). The lower affinity of the GR for cortisol results in its selective activation during circadian and stress induced cortisol zeniths, whilst the MR is activated also during GC-nadirs (Conway-Campbell et al., 2007). The imbalance between the two receptors has been implicated in

Abbreviations: amg, amygdala; CAS (u), upper bank of the calcarine sulcus; CAS (l), lower bank of the calcarine sulcus; Cb, cerebellum; CG, cingulate gyrus; CN, caudate nucleus; DG, dentate gyrus; GC, glucocorticoid; GP, globus pallidus; GR, glucocorticoid receptor; Hi(CA1-3), cornu ammonis of the hippocampus subregions 1-3; Hi(CA4), cornu ammonis of the hippocampus subregions 4; IFG, inferior frontal gyrus; ITG, inferior temporal gyrus; LC, locus coeruleus; MFG, middle frontal gyrus; MR, mineralocorticoid receptor; MTG, middle temporal gyrus; NA, nucleus accumbens; PG, pineal gland; PHG, parahypocampal gyrus; Pit, pituitary gland; Put, putamen; PVN, paraventricular nucleus; SFG, superior frontal gyrus; SN, substantia nigra; SOG, superior occipital gyrus; SPG, superior parietal gyrus; SUBv-sp, ventral subiculum pyramidal layer; WM, white matter; VMHvl, ventromedial hypothalamus (ventrolateral).

* Corresponding author. Institute of Immunology, Centre de Recherche Public de la Santé, Laboratoire National de Santé, 20A rue Auguste Lumière, L-1950 Luxembourg, Luxembourg. Tel.: +352 490604 220; fax: +352 490686.
E-mail address: claude.muller@CRP-SANTE.LU (C.P. Muller).

0022-3956/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jpsychires.2013.07.022

the vulnerability to disease, especially those associated with an impaired or disfunctional HPA axis such as in major depressive disorder (MDD) (De Kloet et al., 1998). In the rodent brain, MR was highly expressed in the limbic system, including the hippocampus (Kretz et al., 2001); whilst GRs are ubiquitously expressed, with the highest density in hypothalamic CRH neurons and pituitary corticotrophs (Morimoto et al., 1996; Reul and de Kloet, 1985). GR levels are considered to be critical for an adequate response to stress.

The human *GR* (OMIM +138040; *NR3C1*), located within chromosome 5, contains eight constant exons (exon 2–9) and nine untranslated alternative first exons 1A–1J with differential tissue-specific expression patterns (Breslin et al., 2001; Presul et al., 2007; Turner and Muller, 2005). Exon 1A and 1I are under the control of a distal promoter region, and the proximal exons 1B–1H and 1J are controlled by individual proximal promoters (Cao-Lei et al., 2011) located in a 3 kp long upstream CpG island that has a high sequence homology between mice, rats and humans (Bockmuhl et al., 2011; McCormick et al., 2000; Turner and Muller, 2005). The mRNA is subsequently spliced at the 3′ end, generating three transcripts encoding the GRα, GRβ and GR-P isoforms. GRα and GRβ are generated by two alternatively spliced exon 9s (9α and 9β), encoding the C-terminal ligand binding domain (LBD). GRα comprising 777 amino acids, is the most active form of the receptor mediating most genomic GC effects. Whilst the exact influence of the 5′ region on the 3′ splicing is unknown, the alternative first exons have been reported to influence splicing and the resulting isoform (Alt et al., 2010; Russcher et al, 2007).

The activity of all the proximal CpG island promoters has been shown to be regulated by DNA methylation (Cao-Lei et al., 2011), although promoter 1F has received the most attention (Alt et al., 2010; McGowan et al., 2009; Moser et al., 2007; Oberlander et al., 2008; Tyrka et al., 2012). In CpG dinucleotide pairs cytosines are subject to covalent modifications including methylation (5-mC) or hydroxymethylation (5-hmC) and their intermediates 5-car boxylcytosine and 5-formylcytosine associated with the degradation pathway. The principal modifications, 5-mC and 5-hmC, are indistinguishable by conventional mapping methods such as bisulfite sequencing used here or methylation-sensitive restriction enzyme-based approaches. These CpG modifications in both promoter 1F, and its rat ortholog 1_7, are thought to represent a key link between the environment and GR expression. Early life adverse events, such as deficits in maternal care, produced epigenetic changes in promoter 1_7 in rodents. One CpG in promoter 1_7, part of a Ngfi-a binding site is particularly susceptible to modification with levels varying from 0 to 100% (Weaver et al., 2004). However, no modification in promoter 1_7 was reported at this position in maternal separation rats and controls (Daniels et al., 2009), and levels never exceeded 20% in rats with a methylation supplementation diet (Herbeck et al., 2010). In humans, hippocampal CpG modifications of the region orthologous to the rat NGFI-A binding site was equally low (Alt et al., 2010; Moser et al., 2007; Oberlander et al., 2008; Tyrka et al., 2012). However, CpG modifications of a separate NGFI-A binding site from that orthologous to the rat (Weaver et al., 2004) was observed in *post mortem* hippocampi of suicide victims to correlate with prior childhood abuse (McGowan et al., 2009). Thus epigenetic CpG modification of the alternative promoters represents an important interface between the early life environment and GC/GR activity.

The GR has been implicated in processes affecting almost all brain regions, however, since our report of the transcriptional control of the GR via its 7 promoters there has been no detailed study of the expression of these transcript variants throughout the human brain. Here, we have examined this transcriptional regulation, as well as the CpG modifications in the associated promoters in human *post mortem* tissues representing 27 brain regions as well as the pituitary gland. Detailed analysis of CpG modification patterns showed that this epigenetic mechanism plays an important role in fine-tuning GR expression throughout the brain.

2. Materials and methods

2.1. Subjects

Post mortem brain tissues were obtained from five donors from a small, well defined, geographic location in and around Chang Mai in Northern Thailand, with no known underlying disease who had been hospitalized after accidents excluding head or brain injuries to which they eventually succumbed; two of which were victims of car accidents, two died due to the blunt chest injury or gunshot respectively, and one has no information about the type of trauma. The patients died at the Intensive Care Unit of the Chiang Mai University Medical Hospital. Brain autopsies were performed for medical or forensic analysis after informed consent of the closest relatives. Informed consent was also given for the use of anonymized brain tissues and clinical and pathological information for research purposes (Table 1). To minimize alterations to DNA modifications autopsies were performed on fresh, unfixed material within 2–10 h after death. The pH of the CSF was in the range 6.8–7.3 for all donors. The brain was cut into, one centimeter autopsy sections and 5 mm diameter punch biopsies were obtained for each of the 28 brain regions (Supplementary Table 1). Samples were immediately placed into at least 5 volumes of RNA*later* (Qiagen) to stabilize mRNA and care was taken that all biopsies were completely submerge by the reagent. The hippocampus (CA1–CA3) was not available for one donor (01). Samples were stored at −20 °C until further analysis. The study was approved by the Ethical Committee of the Faculty of Medicine, Chiang Mai University, Thailand.

2.2. DNA/RNA extraction

A 1.5 mm^3 cube was excised from each sample and both genomic DNA and total RNA were extracted using AllPrep DNA/RNA mini kit (Qiagen, Venlo, Netherlands) following the manufacturer's protocol. Genomic DNA and total RNA concentration were measured on a NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Rockland, DE, USA). Genomic DNA was stored at −20 °C and used for bisulfite modification and pyrosequencing. RNA was stored at −80 °C and used for real-time quantitative RT-PCR.

2.3. cDNA synthesis

Synthesis of cDNA was carried out at 50 °C for 60 min using 200 U Superscript III RT (Invitrogen, Merelbeke, Belgium) and 2.5 mM dT20 primer in a 50 μl reaction containing 250 mM Tris–HCl, 375 mM KCl, 15 mM $MgCl_2$, 10 mM dithiothreitol and 500 mM deoxynucleoside triphosphates (dNTPs). A negative

Table 1
Clinicopathological information.

Number	Gender	Age (year)	Clock time of death	Post-mortem delay (h:min)	Cause of trauma
01	male	54	00:20	10:10	
02	male	43	03:00	7:00	Blunt chest injury
03	female	59	12:00	3:30	Car accident
04	male	24	03:10	9:50	Car accident
05	male	20	05:30	7:00	Gun shot
Mean ± SD		40 (17.4)		7:30 (2:25)	