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Glucocorticoid receptor protein expression in human hippocampus; stability with age

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ABSTRACT

The glucocorticoid receptor (GR) exerts numerous functions in the body and brain. In the brain, it has been implicated, amongst others, in feedback regulation of the hypothalamic-pituitary-adrenal axis, with potential deficits during aging and in depression. GRs are abundantly expressed in the hippocampus of rodent, except for the Ammon's horn (CA) 3 subregion. In rhesus monkey however, GR protein was largely absent from all hippocampal subregions, which prompted us to investigate its distribution in human hippocampus. After validation of antibody specificity, we investigated GRα protein distribution in the postmortem hippocampus of 26 human control subjects (1–98 years of age) and quantified changes with age and sex. In contrast to monkey, abundant GR-immunoreactivity was present in nuclei of almost all neurons of the hippocampal CA subfields and dentate gyrus (DG), although neurons of the CA3 subregion displayed lower levels of immunoreactivity. Colocalization with glial fibrillary acidic protein confirmed that GR was additionally expressed in approximately 50% of the astrocytes in the CA regions, with lower levels of colocalization (approximately 20%) in the DG. With increased age, GR expression remained stable in the CA regions in both sexes, whereas a significant negative correlation was found with age only in the DG of females. Thus, in contrast to the very low levels previously reported in monkey, GR protein is prominently expressed in human hippocampus, indicating that this region can form an important target for corticosteroid effects in human.

1. Introduction

Glucocorticoid hormones (GCs) are important mediators of the stress response in mammals, including humans. Exposure to a stressor triggers activation of corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus (PVN) of the hypothalamus, which eventually induces GC release from the adrenal gland. GCs are highly lipophylic transcription factors that exert numerous effects on metabolism, inflammation, and cognition (de Kloet et al., 2005). When in the circulation, GCs exert negative feedback inhibition on the same regions that triggered their initial release (i.e., the hypothalamus and pituitary), and they also influence behavioral adaptation. These latter effects are generally thought to be mediated, at least partly, through the low affinity glucocorticoid receptor (GR) that is abundantly expressed in the hippocampus of rodents (Reul and de Kloet, 1985). The hippocampal formation is a key limbic region that participates in spatial navigation and the modulation of cognition, mood, and behavior (de Kloet et al., 2005). Based on the presence of GR and the results from various pharmacological studies it has been postulated that the hippocampus is an area in which GCs act to modulate behavior and hypothalamo-pituitary-adrenal (HPA) axis activity (Jacobson and Sapolsky, 1991; Juruena et al., 2006; Sapolsky et al., 1984).

The GR is a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors. After ligand binding, the hormone receptor complex translocates from the cytoplasm to the nucleus where it influences gene transcription.

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In rodents, GR is the dominant receptor that mediates effects of stress levels of GCs, and helps to maintain GC levels within specific limits (Erdmann et al., 2008; Kretz et al., 1999). Aberrant GR expression has been implicated in stress resistance, anxiety, and depression (Alt et al., 2010; de Kloet et al., 2005; Ridder et al., 2005; Wang et al., 2012; Wei et al., 2007). In humans, there is a considerably diversity of GR transcripts and isoforms (Sinclair et al. 2011), which includes 13 exon 1 messenger RNA variants and 8 N-terminal variants, which arise from the predominant GR isoform, GRα, that differ in size from 94 to 54 kDa based on the location of their translation start site.

The regional distribution of the GR protein in the hippocampus has been studied before in various species. In rodents, GR expression is ubiquitous throughout the brain and enriched in key regions of the HPA axis and the hippocampus, and GR is abundantly expressed in the CA pyramidal cell layer and in the granule cell layer of the dentate gyrus (DG), with generally lower levels in the CA3 subregion (Morimoto et al., 1996; Sarabdjitsingh et al., 2010).

In contrast to this well-established distribution in rodent brain, distribution of GR protein in the primate hippocampus is poorly studied. To date, there are no detailed studies of regional protein expression of the GR in the human hippocampus. In rhesus monkey, a general absence of GR was reported in the main neuronal layers of the hippocampal formation, and astroglia did express GR (Sanchez et al., 2000). Because this suggested a species difference in GR expression, we set out to study GR protein distribution in the human hippocampus and hypothalamus.

To validate our antibody and address the influence of postmortem delay on GR expression, we studied a series of postmortem rat brains, performed antigen preadsorption and Western blot, and studied GR coexpression in CRH-containing parvocellular neurons of the human hypothalamic PVN, an important nucleus for GR-mediated feedback inhibition, that also expresses vasopressin, and is involved in HPA feedback inhibition, and as such, is expected to at least express significant levels of GR protein (Erkut et al., 1998; Han et al., 2005; Kretz et al., 1999; Lucassen et al., 1994; Uht et al., 1988; Wang et al., 2008). We next questioned whether GR expression in the human hippocampus is comparable with the distribution in the rodent or primate hippocampus. Third, considering the numerous reports on changes with age (Bao and Swaab, 2007; Bizon et al., 2001; Hassan et al., 1999; Mizoguchi et al., 2009; Murphy et al., 2002; Perlman et al., 2007; Raadsheer et al., 1993; Sinclair et al., 2011; van Eekelen et al., 1992), we investigated whether age- and sex-related changes in GR protein occur in a cohort of 26 control subjects ranging from 1 to 98 years of age.

2. Methods

2.1. Human brain tissue

Postmortem human brain tissue used in this study was obtained from the Netherlands Brain Bank (NBB) and written permission was obtained from the patients or the next of kin for all brain autopsies and for the use of the tissues and clinical data for research purposes. Twenty-six subjects were studied, 12 male and 14 female, ranging in age from 1 to 98 years. Clinicopathological details on disease condition, cause of death, pH of cerebrospinal fluid, and postmortem delay (PMD) are given in Table 1.

The hippocampus proper was dissected at autopsy, fixed in formalin for 1–2 months, dehydrated, embedded in paraffin, and serially sectioned at 8 μm. Midlevel sections of each subject were used for immunohistochemistry for GR. To study whether GR-immunoreactivity (ir) is distributed in a homogenous way along the septotemporal axis, 3 groups of 4 additional patients were

Table 1
Clinicopathological data of the human control cases

Brain number	Age (y)	Sex	PMD (h:min)	pH	Brain region	Cause of death	Medication
93-006	1	M	5:30	NA	Hippocampus	Unclear; possible viral encephalitis	NA
B.A	2	F	12:00	NA	Hippocampus	Wilms tumor	NA
B	2	F	18:00	NA	Hippocampus	Pneumonia, sepsis	NA
G.D	3	F	24:00	NA	Hippocampus	Cardiorespiratory insufficiency	NA
P	8	F	8:00	NA	Hippocampus	Acute lymphoid leukemia	NA
F.A	11	F	6:00	NA	Hippocampus	Acute lymphoid leukemia	NA
00-141	20	F	8:45	6.50	Hippocampus	Sudden death	NA
97-159	48	M	5:30	6.88	Hippocampus	Legal euthanasia	Midazolam
05-034	56	M	14:00	7.03	Hippocampus	Terminal congestive heart failure	NA
05-068	56	M	9:15	6.54	Hippocampus	Myocardial infarction	Domperidone
98-127	56	M	5:25	6.55	Hippocampus	Myocardial infarction	Ceftriaxone
90-065	57	M	4:25	8.05	Hippocampus	Myocardial infarction	NA
01-004	64	F	8:35	6.40	Hippocampus	Myocardial infarction	Tramadol
97-042	65	F	12:50	6.94	Hippocampus	Myocardial infarction	Adrenaline
06-037	66	M	7:45	6.70	Hippocampus	Ruptured abdominal aneurysm aorta	Testosteron
03-054	67	M	4:30	NA	Hippocampus	Cardiac shock/multiple organ failure	Insulin
04-015	69	F	4:20	6.12	Hippocampus	Cardial decompensation	NA
95-072	75	M	7:15	6.33	Hippocampus	Myocardial infarction	Digoxine
03-013	82	F	11:30	6.77	Hippocampus	Congestive cardiac failure	Trimethoprim
07-007	84	M	5:35	6.98	Hippocampus	Myocardial infarction	Midazolam, morphine
05-017	87	M	10:20	6.32	Hippocampus	Pneumonia, heart failure	NA
96-078	87	F	8:00	6.91	Hippocampus	Myocardial infarction	Codeine
93-035	89	F	4:20	6.68	Hippocampus	Myocardial infarction	Morphine
01-006	91	F	5:45	6.49	Hippocampus	Sudden death	Morphine, digoxine
05-063	93	F	4:00	6.64	Hippocampus	Myocardial infarction	Midazolam, morphine
00-027	98	M	8:40	6.66	Hippocampus	Cardiac tamponade	NA
96-411	49	M	19:00	NA	Hypothalamus	Cardio-pulmonal insufficiency	NA
98-200	46	F	11:00	NA	Hypothalamus	NA	NA
S09-015[a]	73	M	6:00	PMD	Hippocampus	Myocardial infarction	NA
S09-061[a]	72	F	7:00	PMD	Hippocampus	Broncopneumonia/myocardial infarction	NA
T08-16837[a]	28	F	0:00	Surgical	Cortex	Temporal lobe epilepsy	NA
T09-1122[a]	35	F	0:00	Surgical	Cortex	Temporal lobe epilepsy	NA

Key: NA, unknown or not available; PMD, postmortem delay.
[a] Frozen human tissue.