The transcription of steroidogenic genes in the human cerebellum and hippocampus: a comparative survey of normal and Alzheimer's tissue

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Abstract

Steroid actions on brain tissue have been implicated in processes such as blood pressure regulation and neurodegeneration, including the progression of Alzheimer's disease (AD). mRNAs from all of the genes required for *de novo* synthesis from cholesterol of aldosterone and corticosterone (equivalent to cortisol in humans) have been identified in rat brain, together with abundant steroid hormone receptors, but the situation in human brain requires clarification. We used real-time RT-PCR to assess whether transcription of 13 steroid-associated genes occurs in human hippocampus and cerebellum, and to identify whether transcription of these genes is significantly altered in cases of AD. Frozen post-mortem samples of hippocampus and cerebellum from patients with AD ($n=7$) and age-matched controls free from neurological disease at the time of death ($n=9$) were used. We found all of the genes under investigation to be transcribed within normal and AD hippocampus and cerebellum except for CYP11B1 (11β-hydroxylase), CYP11B2 (aldosterone synthase) and CYP17 (17α-hydroxylase). No significant differences in mRNA levels were observed between the AD tissue and the equivalent control tissue, although significant regional differences in gene transcription were observed between hippocampus and cerebellum in AD and control samples. The absence of key mRNAs from human hippocampus and cerebellum rules out the *de novo* generation of aldosterone, cortisol or the sex steroids within these regions. However, the pattern of gene expression does suggest that the mineralocorticoid 11-deoxycorticosterone can be generated *de novo*. There is no evidence of a link between AD and altered steroid biosynthesis within human hippocampus and cerebellum.

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Introduction

The tissue expression of genes encoding steroidogenic enzymes is more widespread than was originally thought. Adrenal cortex, ovaries and testes are clearly the major sites of steroidogenesis, but studies have now started to examine tissues such as the heart, the vasculature and the central nervous system (CNS; Davies & MacKenzie 2003). Of those so-called local steroidogenic systems, the data in relation to the CNS are most compelling. Thus, extensive study of the rat brain has detected low-level transcription of all of the genes required in the synthesis of aldosterone, the major mineralocorticoid, and corticosterone, the major rodent glucocorticoid (equivalent to cortisol in humans; Mellon & Deschepper 1993, Strömstedt & Waterman 1995, Erdmann *et al.* 1996, Gòmez Sánchez *et al.* 1996, 1997, Furukawa *et al.* 1998). In addition to transcription, we have previously demonstrated that aldosterone synthase and 11β-hydroxylase are themselves present in the rat brain and that they are found at particularly high levels within the neurons of the hippocampus and cerebellum (MacKenzie *et al.* 2000*a*,*b*). Furthermore, we were able to show that the aldosterone synthase gene, CYP11B2, is subject to transcriptional regulation by dietary sodium within the rat hippocampus and cerebellum (Ye *et al.* 2003). Taken together, these studies point to a complete steroidogenic system under regulatory control distinct from that of the adrenal cortex.

Parallel studies showed that aldosterone and corticosterone are capable of exerting significant physiological effects in the CNS. This was not in itself surprising: parts of the brain are rich in the mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs; Reul & de Kloet 1986, Agarwal *et al.* 1993). Intracerebroventricular (i.c.v.) administration of aldosterone to the rat increases systemic blood pressure at doses too low to induce an effect when administered subcutaneously (Gòmez Sánchez 1986). Co-infusion of corticosterone or the MR antagonist RU-28318 together with the i.c.v. aldosterone attenuates this hypertensive effect (Gòmez Sánchez *et al.* 1990*a*,*b*).

Glucocorticoids influence cognitive function, depression and neurodegeneration (Herbert *et al.* 2006), and human subjects with Cushing's syndrome are known to have a reduced hippocampal volume (Sapolsky 2000). This evidence suggests that glucocorticoids could play a role in the

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pathogenesis of Alzheimer's disease (AD), possibly by accelerating hippocampal damage. However, direct evidence from patients with AD is inconclusive, while some studies have found them to be mildly hypercortisolic when compared with a non-demented control group (Davis *et al.* 1986, Maeda *et al.* 1991, Umegaki *et al.* 2000), others have not (Ferrier *et al.* 1988). Furthermore, many patients who are hypercortisolic due to Cushing's syndrome, depression or the administration of synthetic glucocorticoids do not develop AD (Swaab *et al.* 2005). Cortisol status in such studies is predominantly assessed by plasma concentration, the index of adrenocortical secretion rate. The results indicate that cortisol from this source may not be an aetiological factor. However, as mentioned previously, it is clear that certain other tissues, including the rat brain, are capable of synthesising glucocorticoid *de novo*, although further investigation of the human brain is required.

Several of the genes involved with adrenal corticosteroidogenesis also participate in the production of the sex steroids, which are implicated in certain brain functions. Experimental work on animals has shown that oestrogen has potent neuroprotective properties and is crucial to the development, maintenance and function of normal neural structures (Turgeon *et al.* 2006). AD is more prevalent in women than in men, even taking into account their greater lifespan (Jorm *et al.* 1994). This may be linked to the post-menopausal decline in oestrogen; oestrogen replacement therapy appears to lower the risk of developing AD in post-menopausal women, but this finding is controversial (Almeida & Flicker 2005). However, synthesis of oestrogen could be an important factor in the protection of injured neurons (Hiltunen *et al.* 2006).

For this study, therefore, we conducted a survey of local steroidogenic gene transcription within normal hippocampus and cerebellum. These regions were chosen because our previous studies showed them to be the areas of highest steroidogenic enzyme expression in the rat brain (MacKenzie *et al.* 2000*a*) and because of the pathophysiological relevance of the hippocampus to AD. We gained access to a local archive of frozen normal and AD hippocampus and cerebellum tissue and then used a quantitative real-time RT-PCR protocol to analyse the transcription of 13 genes associated with steroidogenesis within these tissues (See Fig. 1; Payne & Hales 2004). Although previous studies have examined and detected the transcription of some of these genes in the human brain, they tended to concentrate on single genes (Stoffel-Wagner *et al.* 1999*a*, Watzka *et al.* 1999, Beyenburg *et al.* 2001) or used pooled RNA samples from commercial sources (Yu *et al.* 2002). This is the first time, to our knowledge, that a comprehensive quantitative survey of steroidogenic gene transcription in the human CNS has been performed, using a common methodological approach in the same set of individual samples, permitting direct comparison of control and AD transcription.

Materials and Methods

Experimental subjects

Following ethical approval of the project by the local ethics committee, samples of hippocampus and cerebellum were taken from frozen blocks previously dissected from fresh whole brain and stored at −80 °C. Control tissue was from patients dying in hospital who had no known history of neurological or psychiatric disease. This tissue was also examined neuropathologically to confirm that it was free of pathology. The inclusion criterion for AD tissue was clinical diagnosis; there was a subsequent neuropathological examination to confirm this. All neuropathology was performed in the Department of Neuropathology, South Glasgow University Hospital NHS Trust as part of the diagnostic assessment of each case. Where possible, tissue samples that had been subject to a relatively short post-mortem delay between death and freezing were selected and in all cases tissue had a post-mortem interval of <24 h; AD brain samples frozen within this period have been shown not to vary greatly in terms of RNA quality (Gutala & Reddy 2004). For this study, nine control samples (age $81{\cdot}3\pm9{\cdot}2$ years, post-mortem delay $10{\cdot}8\pm8{\cdot}2$ h) and seven AD samples (age $86{\cdot}1\pm7{\cdot}3$ years, post-mortem delay $6{\cdot}6\pm4{\cdot}5$ h) were used (mean ± S.E.M).

RNA isolation and cDNA preparation

Total RNA was isolated from frozen brain tissue using the RNeasy Lipid Tissue Mini Kit (Qiagen) standard protocol and DNased using the TURBO DNA-free kit (Ambion (Europe) Ltd, Huntingdon, UK). The RNA quality was assessed using the 2100 Bioanalyzer (Agilent Technologies UK Ltd, Stockport, UK) that provides an objective measure of the RNA quality termed the RNA integrity number (RIN). Samples with RINs $>5{\cdot}5$ are deemed suitable for use in real-time RT-PCR studies (Schroeder *et al.* 2006), and all of the samples used in this study had RINs >7. No correlation was observed between the post-mortem delay of the tissue and the RIN of its isolated RNA (data not shown). RNA was converted to cDNA using the ImProm-II Reverse Transcription System (Promega UK Ltd) standard protocol with random primers ($0{\cdot}5$ μg/reaction), $6{\cdot}0$ mM $MgCl_2$ and 500 ng total RNA per reaction. All samples were reverse transcribed alongside an equivalent reaction omitting reverse transcriptase (−RT control) and water blanks. Adrenal gland cDNA was prepared in the same way using commercially sourced unpooled human adrenal gland total RNA (Ambion) that had also been subjected to DNase treatment. All reactions had a final volume of 20 μl and this was diluted to 100 μl with water to provide template for the real-time PCR.

Real-time PCR assay development

The genes studied included those responsible for adrenal corticosteroidogenesis (Payne & Hales 2004): CYP11A1 (encoding the side-chain cleavage enzyme), 3β-hydroxysteroid

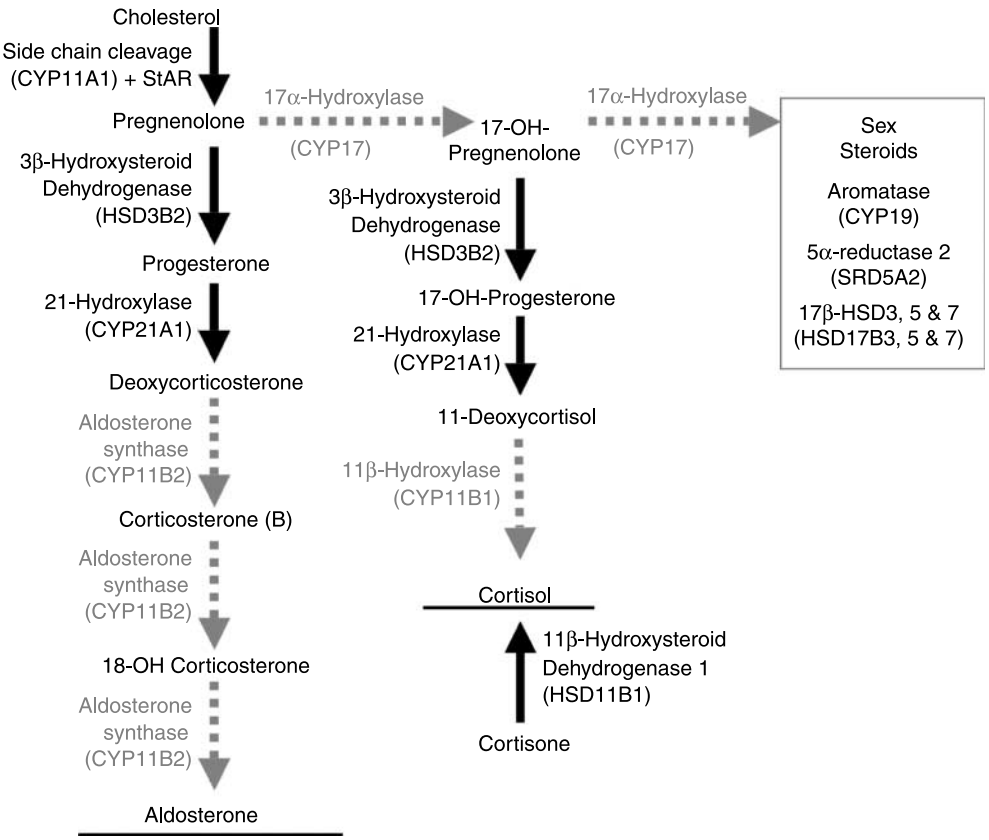

Figure 1 The biosynthetic pathway for corticosteroids, androgens and oestrogens. Enzymes are shown with their genes' names in brackets; all of the genes shown were investigated for this study. Enzymes/genes whose mRNA was detected in human hippocampus and cerebellum as a result of this study are shown in black text next to solid arrows; undetected enzyme/gene names are shown in grey next to broken arrows.

dehydrogenase (HSD) type 2 (HSD3B2), CYP21B (21-hydroxylase), CYP17 (17α-hydroxylase), CYP11B1 (11β-hydroxylase), CYP11B2 (aldosterone synthase) and the Steroidogenic Acute Regulatory Protein (StAR), responsible for the initial rate-limiting step of transporting cholesterol to the side-chain cleavage enzyme). We also examined several of the genes involved in sex steroid biosynthesis including CYP19 (aromatase) and SRD5A2 (5α-reductase type 2) as well as 17β-HSD3, 17β-HSD5 and 17β-HSD7 (HSD17B 3, 5 and 7 respectively). Finally, we looked at expression of the *HSD11B1* gene whose product, the 11β-HSD1 enzyme, is responsible for regenerating active cortisol or corticosterone from the inactive metabolites cortisone or 11-dehydrocorticosterone, thereby increasing local levels of glucocorticoid; this gene has previously been linked to increased risk of AD (de Quervain *et al.* 2003).

Real-time PCR assays for use with the Human Universal ProbeLibrary Set (Roche Applied Science) were developed for each of the target genes and the GAPDH reference gene. Primer pairs were designed with the aid of the Roche Universal Probe Library Assay Design Center (www.roche-applied-science.com) and checked for specificity using NCBI BLAST (http://www.ncbi.nlm.nih.gov/BLAST); primers with no significant similarity to other loci were selected (See Table 1) and synthesised (MWG Biotech AG, Ebersberg, Germany). Primers were then tested using the real-time PCR protocol (see below) and the efficiency of each primer pair calculated using serial tenfold dilutions of adrenal gland, hippocampus or cerebellum cDNA (Pfaffl 2001; See Table 1). Due to their extreme sequence similarity, primers for CYP11B1 and CYP11B2 were subjected to further tests using plasmids containing cloned cDNAs for these genes and were found to be specific (data not shown).

Real-time PCR

All reactions were performed in a 384-well plate format on an ABI 7900 HT Prism Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Each reaction contained 100 nM of the relevant Universal Probe (see Table 1), 400 nM of each primer, 1 μl diluted cDNA and 2·5 μl ABsolute QPCR ROX Mix (ABgene, Epsom, UK) in a final volume of 5 μl. Reactions were incubated at 95 °C for 15 min followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. The crossing point (C_t) is the PCR cycle number at which a sample reaches the

Table 1 Summary of the quantitative PCR assays used in this study

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)	UPL probe no.	PCR efficiency
GAPDH	AGC CAC ATC GCT CAG ACA C	GCC CAA TAC GAC CAA ATC C	60	2·01
CYP11A1	AGG AGG GGT GGA CAC GAC	TTG CGT GCC ATC TCA TAC A	59	2·04
StAR	TAC GTG GCT ACT CAG CAT CG	ACC TGG TTG ATG ATG CTC TTG	83	1·70
HSD3B2	AGG CCT TCA GAC CAG AAT TG	CCT TCA AGT ACA GTC AGC TTG G	50	1·99
CYP21B	GAG GGC ACA GTC ATC ATT CC	GCT CCA GGA AGC GAT CAG	14	1·93
CYP11B1	ACT AGG GCC CAT TTT CAG GT	GGC AGC ATC ACA CAC ACC	68	1·93
CYP11B2	AGA TGC ACC AGA CCT TCC AG	GTG GTC CTC CCA AGT TGT ACC	57	2·01
CYP17	CTA TGC TCA TCC CCC ACA AG	TTG TCC ACA GCA AAC TCA CC	67	1·83
HSD11B1	CAA TGG AAG CAT TGT TGT TCG	GGC AGC AAC CAT TGG ATA AG	20	1·70
CYP19	GAA TTC ATG CGA GTC TGG ATC T	TCA TTA TGT GGA ACA TAC TTG AGG A	55	1·98
SRD5A2	CAG CTA CAG GAT TCC ACA AGG	TCA ATG ATC TCA CCG AGG AA	50	1·60
HSD17B3	AGG CCA TTG CCA CAG AGA	TGT CAT CTT TTG TAA AAT CTG CTT G	52	1·60
HSD17B5	CAT TGG GGT GTC AAA CTT CA	CCG GTT GAA ATA CGG ATG AC	27	1·67
HSD17B7	TTG ACA CCA TAT AAT GGA ACA GAA G	TGA TCA GAG GAT TGA GAG ATT CAG	31	1·99

The UPL probe no. refers to the degenerate fluorescent probes supplied in the Universal Probe Library Human Set (Roche Applied Sciences). The calculated PCR efficiencies listed are those used for correction by the relative expression software tool (REST).

threshold level of fluorescence. The C_t is proportional to the amount of target material in any given sample: the lower the C_t, the higher the amount of target material within a sample. The hippocampus, cerebellum and adrenal gland cDNA reactions were run in triplicate and the mean C_t value from the three reactions was used for subsequent data analysis. Blank and −RT control reactions were run alongside the positive samples.

Data analysis

Data were analysed using the relative expression software tool (REST). REST-384 is an Excel spreadsheet-based tool that permits the comparison of numerous target genes' expression using a common reference gene, while taking into account differences in reaction efficiency that are not addressed by the commonly used $\Delta\Delta C_t$ method (Pfaffl 2001, Pfaffl *et al.* 2002). It employs a non-parametric method to assess the statistical significance of any differences between groups, calculating *P* values on the basis of the pair wise fixed reallocation randomisation test which jointly reallocates the C_t values for reference and target genes to control and sample groups, then calculates the resulting expression ratios on the basis of the mean values. In the course of each test 10 000 such randomisations were performed. This method avoids the reduction in power that one encounters with parametric tests and avoids assumptions about data distribution, which might be encountered in non-parametric tests that employ measurements of rank (Pfaffl *et al.* 2002).

All target gene results were adjusted for GAPDH reference gene expression. Although GAPDH transcription has been shown to vary under certain circumstances, it has previously been shown to be a valid reference gene for AD brain studies (Gutala & Reddy 2004) and comparison of GAPDH C_t results from this study showed no significant variation between hippocampus and cerebellum samples, control and AD subjects or between brain and adrenal tissue, thus fulfilling the requirements of a reference gene.

Results

An identical array of the genes under investigation was detected in the hippocampus and cerebellum of both control and AD tissue. CYP11A1, StAR, HSD3B2, CYP21B, HSD11B1, CYP19, SRD5A2, HSD17B3, HSD17B5 and HSD17B7 mRNAs could all be detected in these tissues. However, CYP11B1, CYP11B2 and CYP17 mRNAs were not detected either in control or in AD tissue. The C_t results are summarised in Table 2. All of the transcripts under investigation were detected in the adrenal gland sample apart from HSD17B3 and SRD5A2. All blank and −RT control reactions failed to amplify. Following correction for the GAPDH reference gene and amplification efficiency of the different assays, the REST-384 program compared transcription levels between control and AD hippocampus and also between control and AD cerebellum but did not identify any significant differences (10 000 REST randomisations, $P > 0{\cdot}05$ in all cases).

Comparison of region-specific transcriptional differences between the hippocampus and cerebellum did yield significant results and these are presented in Table 3 (10 000 REST randomisations).

The mRNA of those genes involved in adrenal corticosteroidogenesis was transcribed at much higher levels in the adrenal gland than in either region of the CNS under examination. Although only a single adrenal RNA sample was used in this study, relative levels of adrenal and CNS mRNAs could be calculated using REST-384 and are shown in Table 4. These results show adrenal levels of transcription to be ∼2–5 orders of magnitude greater than those of the CNS.

Table 2 C_t values obtained from real-time RT-PCR of human central nervous system and adrenal RNA. Values are presented as the mean C_t values ±S.E.M. from control samples (n=9), AD samples (n=7) and adrenal sample (n=1)

	Hippocampus		Cerebellum		
	Control	AD	Control	AD	**Adrenal gland**
Gene					
GAPDH	20·59±0·29	21·24±0·48	20·77±0·14	21·44±0·83	20·87
StAR	29·16±0·38	30·34±0·69	30·07±0·16	32·03±0·88	19·25
CYP11A1	29·54±0·24	30·73±0·29	30·03±0·25	30·94±0·65	21·86
HSD3B2	36·32±0·35	35·99±0·59	36·90±0·32	36·46±0·31	22·30
CYP21B	28·91±0·44	29·51±0·32	27·51±0·21	29·46±0·51	21·25
CYP11B1	ND	ND	ND	ND	18·47
CYP11B2	ND	ND	ND	ND	24·82
CYP17	ND	ND	ND	ND	22·54
CYP19	32·80±0·52	33·58±0·36	34·01±0·64	36·27±0·56	24·50
HSD11B1	27·58±0·35	29·45±0·38	24·27±0·39	25·84±0·51	25·80
HSD17B3	27·73±0·43	28·98±0·33	30·05±0·31	31·50±0·50	ND
HSD17B5	30·35±0·33	30·96±0·21	30·19±0·28	32·15±0·50	29·32
HSD17B7	26·10±0·09	27·79±0·26	25·64±0·10	27·57±0·34	26·11
SRD5A2	35·08±0·55	35·43±0·38	31·80±0·30	33·03±0·43	ND

ND, not detected. C_t values were subsequently corrected for GAPDH reference gene amplification and relative amplification efficiency (see Table 1) by the REST-384 program before non-parametric analysis using the pair wise fixed reallocation randomisation test.

Discussion

Steroid production and action within the brain is not a new concept; the term 'neurosteroids' was coined to refer to such steroids and several previous studies have identified a number of the genes required for their production within the human CNS. In this study, our intention was to use a number of individual human brain tissue samples and a single RT-PCR–based method in order to identify which steroids are likely to be generated *de novo* within the normal human hippocampus and cerebellum, as these were the regions which showed greatest steroidogenic gene expression in our previous studies (MacKenzie *et al.* 2000*a*). Furthermore, the quantitative nature of our assay enabled us to assess whether transcription of any of these genes was significantly altered in cases of AD when compared with a control group, but found no evidence of altered steroidogenic gene transcription in cases of AD. Although this does not rule out a role for corticosteroids from other sources, most obviously the adrenal gland, these results suggest that enhanced or diminished local corticosteroidogenesis within the hippocampus and the cerebellum is not a factor in advanced cases of AD, although a role in its early development cannot be ruled out.

In our control and AD tissues, we confirmed the presence of several mRNAs previously detected in these or other regions of the human CNS, including CYP11A1 (previously found in various regions including cerebellum and hippocampus (Watzka *et al.* 1999, Yu *et al.* 2002)), StAR (various regions including cerebellum; hippocampus was not examined (Kim *et al.* 2003)), HSD3B2 (various regions including hippocampus and cerebellum (Yu *et al.* 2002)), CYP21B (various regions including hippocampus and cerebellum (Beyenburg *et al.* 2001, Yu *et al.* 2002)), HSD11B1 (hippocampus, cerebellum and prefrontal cortex (Sandeep *et al.* 2004)), CYP19 (various regions, although cerebellum was not examined (Stoffel-Wagner *et al.* 1999*a*)), HSD17B3 (temporal lobe (Stoffel-Wagner *et al.* 1999*b*)) and HSD17B5 (temporal lobe (Steckelbroeck *et al.* 2001)). Our detection of HSD17B7 mRNA in human CNS is a novel finding. We also detected SRD5A2 transcripts within hippocampus and cerebellum, although a previous study had failed to find it in human hippocampus (Stoffel-Wagner *et al.* 2000), possibly due to a lack of sensitivity in their assay, as our C_t results for this gene were high (~35 cycles in hippocampus), signifying low levels of transcription. Given the use of homogenised brain tissue in this study, it was not possible to assess whether gene transcription is solely neuronal or glial, or whether it occurs in both cell types. Although samples were matched by age, we could not control for factors such as cause of death and duration of terminal illness, which may conceivably have some effect on local steroid production

Table 3 Significant differences in relative mRNA levels between hippocampus and cerebellum

	Control	AD
Gene		
HSD3B2	n.s	H>C 2·3-fold*
CYP21B	C>H 2·0-fold*	n.s
CYP19	n.s	H>C 10·9-fold‡
HSD11B1	C>H 7·9-fold‡	C>H 7·0-fold‡
HSD17B3	H>C 3·7-fold†	H>C 5·7-fold‡
HSD17B5	n.s	H>C 3·2-fold†
SRD5A2	C>H 3·7-fold‡	n.s

Results are shown as fold difference in detected mRNA levels between hippocampus and cerebellum, where H>C denotes a greater level in hippocampus and C>H a greater level in the cerebellum. *P<0·05, †P<0·01, ‡P<0·001, n.s., not significant.

Table 4 Levels of mRNA in human adrenal gland relative to control human cerebellum and hippocampus samples

Gene	Hippocampus	Cerebellum
StAR	$2{\cdot}5\times10^{-3}$	$1{\cdot}5\times10^{-3}$
CYP11A1	$3{\cdot}4\times10^{-3}$	$1{\cdot}9\times10^{-3}$
HSD3B2	$5{\cdot}4\times10^{-5}$	$2{\cdot}9\times10^{-5}$
CYP21B	$5{\cdot}4\times10^{-3}$	$1{\cdot}1\times10^{-2}$

Data are presented as the quantity of mRNA in hippocampus or cerebellum control samples relative to the adrenal RNA sample, where adrenal levels are all equal to 1. The data are based on the comparison of nine control samples with a single commercially sourced human adrenal RNA sample.

as well as circulating steroid levels. Similarly, it was not known which, if any, of the AD or control subjects had been treated with exogenous steroids. In spite of this, steroidogenic gene expression, where detected, was extremely consistent across all samples, as demonstrated in Table 2.

In our previous rat brain studies, CYP11B1 and CYP11B2 transcription was low but consistently detectable in hippocampus and cerebellum. In contrast, no expression was detectable in the human brain, indicating a significant species difference in local steroidogenesis. Yu *et al.* (2002) examined the transcription of several steroidogenic genes in pooled, commercially sourced human brain RNA samples and were similarly unable to detect CYP11B1 and CYP11B2 mRNAs, although they could detect it in other regions they examined, particularly corpus callosum. However, they detected CYP11A1, HSD3B2 and CYP21B transcription in pooled hippocampi and cerebella, in agreement with the findings here. Their detection of CYP17 mRNA contrasts with its absence from any of our hippocampal or cerebellar samples, although Stoffel-Wagner (2003) was similarly unable to detect it. Stoffel-Wagner (2003) also alludes to an unpublished study where CYP11A1 and CYP11B2 mRNAs could not be detected in human hippocampus. The absence of CYP17 transcription would obviate *de novo* synthesis of sex steroids; any synthesis within the CNS would instead have to rely on the conversion of circulating steroid precursors. Age may be a confounding factor; Yu's pooled samples contained 10–70 samples from patients whose ages ranged from 15 to 78 years, whereas our samples were drawn entirely from an older population ranging from 67 to 95 years. Brain expression of steroidogenic genes may be higher in younger people and transcription of CYP11B1, CYP11B2 and CYP17 may become detectable in a younger population. Few data are available on age-related changes; the finding by Watzka *et al.* (1999) of higher hippocampal CYP11A1 transcription in a child when compared with adults was based on a single child sample. On the other hand, in the same study, where a greater number of samples were available, temporal lobe cortex CYP11A1 transcription was significantly lower in children when compared with adults.

Our method was sensitive enough to detect significant differences as low as twofold between CNS regions, and variations between the control and the AD samples were consistent for each region (e.g. an approximately sevenfold higher level of cerebellum HSD11B1 mRNA in both groups). However, it is perhaps surprising that the greatest difference that we identified, an 11-fold higher level of CYP19 mRNA in the AD hippocampus than cerebellum ($P<0{\cdot}001$), was not seen in our control group; this appears to be due to control cerebellum having a lower – but not significantly low – C_t value than AD cerebellum for CYP19 (see Table 2).

CNS transcription of genes involved with corticosteroid synthesis was consistently much lower than that of the adrenal gland. While such low levels of expression could not significantly affect circulating levels of adrenally sourced corticosteroid, the abundance of MR and GR within hippocampus and cerebellum suggest a paracrine or autocrine mode of action for any steroid synthesised within the CNS. RT-PCR of adrenal cDNA also detected transcription of the aromatase (CYP19) gene, confirming the recent study by Baquedano *et al.* (2007) who detected aromatase mRNA in normal adrenal medulla and zona glomerulosa and confirmed the enzyme's expression by immunohistochemistry.

Finally, our findings appear to rule out *de novo* aldosterone biosynthesis within the human hippocampus and cerebellum as no CYP11B2 mRNA was transcribed. *De novo* production of cortisol from cholesterol within these CNS regions is also unlikely, given the absence of 11β-hydroxylase gene expression, although the presence of 11β-HSD1 would permit the modulation of local glucocorticoid levels by regeneration from cortisone. Thus, despite relatively high levels of MR, human hippocampus and cerebellum cannot synthesise the major mineralocorticoid. Indeed, the pattern of gene expression (see Fig. 1) identifies the ultimate likely product as the weaker mineralocorticoid, 11-deoxycorticosterone (DOC). Although DOC has a lower potency than aldosterone (Sharma *et al.* 2006), hippocampal MRs in the human brain are unlikely to be protected from cortisol occupation by significant 11β-HSD2 expression, low levels of locally-synthesised steroid might be able to act in an autocrine or paracrine mode of action. The array of transcription that we demonstrate here suggests that the capacity exists to produce mineralocorticoid within these large, MR-rich brain regions and, as White (2003) observed of the similar situation in the human heart, the physiological implications of this local system warrant further examination. A recent study by Yao *et al.* (2007) has shown that neuron-specific overexpression of the aldosterone synthase gene results in significant increases in systemic blood pressure in transgenic rats. While our study encourages caution when extrapolating results from rodent studies to humans, locally produced mineralocorticoids could be capable of producing real physiological effects and, ultimately, it may be of little importance whether the responsible mineralocorticoid is aldosterone or DOC.

By their nature, RT-PCR studies such as this cannot provide a precise indication of actual enzyme abundance within the hippocampus and cerebellum, or of the true

steroidogenic capacity of these regions. Study of other brain regions or of younger subjects may reveal that the CNS also possesses very different steroidogenic capabilities. Nevertheless, in this study we are able to utilise RNA of high quality from a range of normal and AD tissue samples in order to provide data that confirm and extend the findings of previous investigations into the human hippocampus and cerebellum. In doing so, we have identified DOC as the likely major product of these sizeable brain regions and we propose that this mineralocorticoid may be capable of performing a physiological role within the human CNS.

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