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11β-Hydroxysteroid Dehydrogenase Type 1 Deficiency Prevents Memory Deficits with Aging by Switching from Glucocorticoid Receptor to Mineralocorticoid Receptor-Mediated Cognitive Control

Joyce L. W. Yau, June Noble, and Jonathan R. Seckl

Introduction

Chronic glucocorticoid (GC) hyperscretion associates with age-related cognitive decline in rodents and humans (Meaney et al., 1995; Yau et al., 1995; Lupien et al., 1998). 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) plays a pivotal role in age-related memory deficits. 11β-HSD1 deficient mice are protected from spatial memory impairments with aging, but the underlying mechanisms are unknown. To determine which brain receptors [high-affinity mineralocorticoid receptors (MRs) or low-affinity glucocorticoid receptors (GRs)] are involved, spatial memory was measured in aged 11β-HSD1−/− mice before and during intracerebroventricular infusion (10 d) of spironolactone (MR antagonist) or RU486 (GR antagonist). Aged C57BL/6J control mice showed impaired spatial memory in the Y-maze; this improved with GR blockade, while MR blockade had no effect. In contrast, aged 11β-HSD1−/− mice showed intact spatial memory that became impaired with MR blockade, but not GR blockade. Hippocampal MR and GR mRNA expression and plasma corticosterone levels were not significantly altered with spironolactone or RU486 in either genotype. These data support the notion that 11β-HSD1 deficiency in aging mice leads to lower intracellular GC concentrations in brain, particularly in the hippocampus, which activate predominantly MRs to enhance memory, while in aging C57BL/6J controls, the increased intracellular GCs saturate MRs and activate predominantly GRs, thus impairing memory, an effect reversed by GR blockade.

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while optimally activating memory-supporting actions via MR, by maintaining lower intracellular GC levels in aged mice. The Y-maze with a2hI T I during the 10th day of osmotic mini-pump infusion. Morning tail venesection blood samples were taken (8:30 – 9:30 a.m.) at the levels of the anterior hippocampus and paraventricular nucleus were postfixed and hybridized with [35S]-UTP-labeled cRNA antisense probes transcribed in vitro from cDNA clones encoding rat MR and GR and quantified at the microscopic level as previously described (Yau et al., 1997).

Corticosterone assay. Plasma CORT levels were measured using an in-house radioimmunoassay (RIA) (Al-Dujaili et al., 1981) modified for microtiter plate scintillation proximity assay (GE Healthcare). For brain CORT levels, steroids were extracted by solvolysis from the dissected tissues as described previously (Ebner et al., 2006) with modifications. Tissue homogenates in phosphate buffer were added slowly to 95% ethanol at 30°C, extracts were centrifuged, dried, reconstituted in 40% methanol, and extracted using C18 Sep-Pak cartridges before RIA.

Statistical analysis. Data are expressed as mean ± SEM and were analyzed using either a one-way or two-way ANOVA followed by Scheffé F tests post hoc as appropriate for individual between-group comparisons. The percentage time in the novel arm was compared with the other arms of the Y-maze by Student’s paired t test, as were before and after treatment times in the novel arm. Significance was set at p < 0.05.

Results

Effect of aging and 11β-HSD1 deficiency on plasma and brain corticosterone levels

Basal plasma CORT levels increased with age in both 11β-HSD1−/− and C57BL/6J control mice (F1,28 = 6.3, p < 0.05) (Fig. 1A), consistent with previous reports in rodents and humans (Issa et al., 1990; Yau et al., 1995; Lupien et al., 1998), but there was no effect of genotype. CORT concentrations in the hippocampus, which reflect free CORT from the periphery and intracellular CORT from 11β-HSD1 activity, increased with age in C57BL/6J mice (F1,28 = 5.7, p < 0.005) but not in 11β-HSD1−/− mice (Fig. 1B). Two-factor ANOVA revealed an effect of genotype (F1,28 = 12.2, p < 0.01) and age × genotype interaction (F1,28 = 5.8, p < 0.05). Aged 11β-HSD1−/− hippocampal CORT levels were significantly decreased (by 57%, p < 0.01) compared to aged controls. This reduction in hippocampal CORT was not evident in young 11β-HSD1−/− mice (Fig. 1B). In the cortex, while 11β-HSD1 deficiency had no effect on CORT in young mice, levels in aged 11β-HSD1−/− mice were decreased by 44% (F1,28 = 6.3, p < 0.05) compared to aged controls (Fig. 1C).

Spatial memory of aged 11β-HSD1−/− and C57BL/6J mice before treatment

Baseline spatial memory performances of all aged (24-month-old) C57BL/6J and 11β-HSD1−/− mice were measured before randomization to treatment (vehicle, RU486, spironolac-
The impaired spatial memory of aged C57BL/6J mice was reversed with central GR but not MR blockade. A. Before antagonist treatment, aged C57BL/6J mice (n = 19) spent more time in the novel arm of the Y-maze than other arms following a 1 min ITI, but with a 2 h ITI, they failed to distinguish the novel arm showing impaired spatial memory. B. The impaired spatial memory of aged C57BL/6J mice (n = 7–8/group) before the drug was reversed after 10 d intracerebroventricular RU486, with mice now spending more time in the novel arm than other arms. All values are means ± SEM. *p < 0.05.

Improved spatial memory in aged C57BL/6J mice following central GR blockade

The intracerebroventricular infusion of vehicle had no effect on the impaired spatial memory of aged C57BL/6J mice before the drug, showing the lack of any practice effect (novel arm before and after vehicle, paired t test, p = 0.15), and spironolactone did not affect the already impaired performance, although there was a trend for the mice to spend even less time in the novel arm than before the drug (paired t test, p = 0.10) (Fig. 2B). In contrast, RU486 improved spatial memory, with the mice spending significantly more time in the novel arm (p < 0.05) than in the previously visited arms and than in individual performances before RU486 (p < 0.05) (Fig. 2B).

Impaired spatial memory in aged 11β-HSD1−/− mice following central MR blockade

The maintained spatial memory of aged 11β-HSD1−/− mice in the Y-maze (2 h ITI) before the drug was not altered by intracerebroventricular infusion of vehicle (Fig. 3B). In contrast to aged C57BL/6J controls, RU486 had no impact on spatial memory in aged 11β-HSD1−/− mice with maintained recognition of the novel arm (p < 0.05 compared to other arms) (Fig. 3B). Spatial memory in aged 11β-HSD1−/− mice was, however, impaired by intracerebroventricular spironolactone, with mice failing to recognize the novel arm and spending significantly less time there than before treatment (p < 0.05) (Fig. 3B).

Hippocampal corticosteroid receptor expression and plasma corticosterone levels

An alternative explanation for these findings is that 11β-HSD1 deficiency and/or receptor antagonist administration alters expression of GR and/or MR or plasma CORT levels. However, there was no significant effect of treatment (spironolactone or RU486) on MR or GR mRNA levels in any hippocampal subregion (Fig. 4A, B) or on GR mRNA in the paraventricular nucleus (PVN) of the hypothalamus (Fig. 4C). Basal plasma CORT levels in C57BL/6J and 11β-HSD1−/− mice, respectively, following spironolactone (122 ± 11 nm; 143 ± 28 nm) or RU486 (116 ± 13 nm; 164 ± 10 nm) treatments were not significantly different from vehicle controls (107 ± 14 nm; 143 ± 21 nm), although there was
an effect of genotype ($F_{1,39} = 5.28, p < 0.05$), with significantly higher CORT levels in 11β-HSD1$^{-/-}$ mice treated with RU486.

**Discussion**

The present results show that spatial memory deficits in aged mice are ameliorated by central GR antagonism. In contrast, maintained spatial memory in aged 11β-HSD1$^{-/-}$ mice is reversed by central MR blockade. These data (1) suggest that (i) age-related memory deficits are associated with long-term brain GC excess acting via GR; (ii) such deficits are not due to irreversible molecular and structural changes in the aged brain, at least in mice; (iii) intracerebral CORT levels appear crucial; and (iv) 11β-HSD1 deficiency, by keeping intracerebral CORT levels low with age, allows spatial-memory-enhancing MR-mediated effects to predominate; and (2) afford clear evidence that GR and MR underpin distinct effects on cognition with aging.

The relationships between GCs, their receptors, and cognition are complex. In general, acute elevations of GCs tend to facilitate learning, notably in a fearful context, but impair retrieval (Roozendaal et al., 2006; Abrari et al., 2009) (Roozendaal, 2002). Chronic GC excess impairs hippocampal-dependent spatial/declarative memory formation (Lupien et al., 1997; Conrad, 2010). Here, the cognition-enhancing effect of GR antagonism in aged C57BL/6J mice was marked. Indeed, chronic RU486 from middle age abolishes electrophysiological disturbances found in aged control mouse hippocampal slices (Talmi et al., 1996). However, chronic stress-induced morphological remodeling of prefrontal pyramidal neurons was not reversed with recovery from stress in aged rats (Bloss et al., 2010), suggesting that not all deleterious long-term effects of GCs are reversible in the aged animal. In contrast, in young rats, GR antagonism only modestly facilitates spatial learning in the “stressful” water maze when circulating CORT levels are elevated (Oitzl et al., 1998a,b), perhaps because young rats have lower CORT levels that barely occupy GR and/or have more robust cognition. The lack of effect of GR blockade on spatial memory in aged 11β-HSD1$^{-/-}$ mice suggests that their lower brain CORT levels were insufficient to substantially occupy GR (Ratka et al., 1989). In contrast, MR antagonism impaired spatial memory in aged 11β-HSD1$^{-/-}$ mice, consistent with the importance of MR occupancy in processing of hippocampus-dependent memory (Oitzl and de Kloet, 1992; Conrad et al., 1997; Douma et al., 1998; Yau et al., 1999). Thus the balance of activated MR and GR, which are critical for neuronal excitability, stress responsiveness, and behavioral adaptation (De Kloet et al., 1998), appears shifted from predominantly GR effects in aged controls to predominantly MR actions in aged 11β-HSD1$^{-/-}$ mice. Many hippocampal GC-responsive genes are regulated either by MR or GR, with fewer genes responsive to both (Datson et al., 2001), supporting the notion that distinct pathways underpin the effects of the antagonists seen here.

11β-HSD1 deficiency did not affect the expression of hippocampal MR and GR or PVN GR mRNAs in aged mice despite the decreased intracerebral CORT levels. This contrasts with increased GR mRNA in hippocampus and PVN of young 11β-HSD1$^{-/-}$ mice on the same strain background (Carter et al., 2009), which plausibly compensates for the lack of intracellular CORT regeneration by increasing cellular responsiveness. This mechanism appears lost in aged animals. The parallel rise in blood CORT levels in both genotypes with aging indicates that such implied reduced plasticity of GR, which occurs in normally aging rodents (Eldridge et al., 1989a,b), might contribute to the GC excess with aging. Moreover, in the aged mice of either genotype, continuous antagonism of brain MR or GR did not alter CORT levels. While acute blockade of brain MR increases basal circulating CORT levels in rats (Ratka et al., 1989; van Haarst et al., 1997), chronic blockade of brain MR had no effect on basal plasma CORT levels in young rats (Yau et al., 1999), as in our aged mice, suggesting that adaptations in the HPA axis have occurred. In contrast, acute blockade of brain GR has been shown to increase (van Haarst et al., 1997) or have no effect on (Ratka et al., 1989) plasma CORT levels, while chronic blockade of brain GR had no effect on basal morning plasma CORT levels (van Haarst et al., 1996), consistent with our present observation, but increased circadian peak levels of CORT (van Haarst et al., 1996). Although plasma CORT levels showed a trend to be higher in aged 11β-HSD1$^{-/-}$ mice than in aged C57BL/6J controls, this was only significant in the RU486-treated animals. However, the effects of intracerebroventricular RU486 in the aged 11β-HSD1$^{-/-}$ mice were not a consequence of the higher plasma CORT per se, since spatial memory was unaffected compared to before treatment.

These findings suggest that decreasing GR activation/increasing MR activation, either by reducing brain GC levels with a selective 11β-HSD1 inhibitor or by GR antagonism, may ameliorate memory impairments even in already aged individuals. The
long-term use of GR antagonists in humans may be problematic, since chronic administration of RU486 (mifepristone) produces generalized GC resistance with high levels of cortisol in compensation (Bamberger and Chrousos, 1995). Perhaps intermittent therapy might exert net cognitive benefits. The use of selective 11β-HSD1 inhibitors to reduce the levels of GC in the aged brain, to reverse the balance of receptor activation from predominantly GR to predominantly MR, appears promising. Indeed, an 11β-HSD inhibitor improved aspects of cognitive function in elderly men and patients with type 2 diabetes without increasing HPA activity (Sandee et al., 2004). Moreover, short-term treatment of already aged control mice with a CNS-active selective 11β-HSD1 inhibitor improves spatial memory over days (Sooy et al., 2010).

References


