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11β-Hydroxysteroid Dehydrogenase Type 1 Activity in Medial Vestibular Nucleus and Cerebellum after Unilateral Vestibular Deafferentation in the Rat

CLARE GUILDING[a], JONATHAN R. SECKL[b] and MAYANK B. DUTIA[a,*]

[a]*Biomedical Sciences, Hugh Robson Building, George Square, Edinburgh EH8 9XD, UK;* [b]*Endocrinology Unit, Edinburgh University, Molecular Medicine Centre, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK*

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In the early stages of vestibular compensation (VC) (the behavioural recovery that follows unilateral vestibular deafferentation), neurons in the medial vestibular nucleus (MVN) on the lesioned side develop a sustained up-regulation of their intrinsic excitability. This plasticity is dependent on the activation of glucocorticoid receptors, which presumably occurs during the acute stress response that accompanies the vestibular deafferentation symptoms. Recent studies have established that the access of glucocorticoids to their intracellular receptors in brain is potently modulated by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), which catalyses the generation of active glucocorticoids from their inert 11-keto forms. In this study, we investigated the presence of 11β-HSD1 bioactivity, and possible changes in activity in the early stage after vestibular deafferentation, in the cerebellar nodulus and uvula, the flocculus/paraflocculus (F/PF) complex and the MVN of the rat. 11β-HSD1 activity was found in each of these brain areas, with especially high levels of activity in the F/PF complex. No differences were found in the level of 11β-HSD1 activity in these brain areas between control rats, sham-operated rats and rats that underwent VC for 4 h after unilateral vestibular deafferentation. These findings demonstrate 11β-HSD1 bioactivity in the MVN and vestibulocerebellum, but exclude the possibility that changes in 11β-HSD1 activity occur in the early period after deafferentation, over the time when changes in MVN neuronal properties take place.

Keywords: 11β-HSD; Cerebellum; Corticosterone; Glucocorticoids; Vestibular compensation; Plasticity

INTRODUCTION

"Vestibular compensation", the behavioural recovery that follows damage to the vestibular receptors of one inner ear or the vestibular nerve, is an attractive experimental model of deafferentation-induced plasticity in the adult brain. Of particular interest is the finding that vestibular compensation (VC), like other forms of brain plasticity, is significantly modulated by behavioural stress and stress-related corticosteroids (Yamanaka *et al.*, 1995; Cameron and Dutia, 1999; Seemungal *et al.*, 2001). Vestibular dysfunction is itself a potent natural stressor; various forms of vestibular challenge (low-frequency motion, Meniere's episodes, vertigo, motion sickness) are to varying degrees stressful (Yardley *et al.*, 1994; Seemungal *et al.*, 2001). After unilateral labyrinthectomy (UL), activation of the hypothalamo-pituitary-adrenal stress axis is indicated by high levels of Fos immunolabelling in the paraventricular nucleus of the rat hypothalamus (Cameron and Dutia, 1999) and by an elevation of salivary levels of cortisol in the guinea pig (Gliddon *et al.*, 2003). Recent findings have directly implicated stress, glucocorticoids and glucocorticoid receptors (GRs) in vestibular plasticity; acute stress or administration of a GR agonist accelerates behavioural recovery after UL while administration of a GR antagonist retards it (Yamanaka *et al.*, 1995; Yamamoto *et al.*, 2000; Seemungal *et al.*, 2001). However, an optimal level of GR activation appears to be needed to facilitate VC, since additional restraint stress applied to a compensating animal also retards behavioural recovery (Yamamoto *et al.*, 2000). At the cellular level, GR activation is necessary for the increase in excitability of ipsi-lesional medial vestibular nucleus (MVN) neurons at 4 h post-UL (Cameron and Dutia, 1999; Johnston *et al.*, 2002). Glucocorticoids exert a wide

*Corresponding author. Tel.: +44-131-650-3252. E-mail: m.b.dutia@ed.ac.uk

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range of actions within the CNS affecting neuronal survival, electrophysiological activity and synaptic transmission (Joels, 1997), but the mechanisms by which GR activation facilitates VC are unknown.

For genomic actions of glucocorticoids, the responsiveness of neurons is determined by cellular levels of expression of GR (and in some sites mineralocorticoid receptors, MR), as well as the relative expression of the pre-receptor modulatory 11β-hydroxysteroid dehydrogenase (11β-HSD) enzymes types 1 and 2 which, respectively, potently activate or deactivate circulating glucocorticoids (Seckl, 1997; Seckl and Walker, 2001). The 11β-HSD enzymes catalyse the interconversion of inert 11-keto glucocorticoids (11-dehydrocorticosterone in rat) and their active 11-hydroxy glucocorticoid forms (corticosterone in rat). 11β-HSD type 2 is an NAD dependant 11β-dehydrogenase that rapidly inactivates corticosterone in rat, and is expressed only in a few discrete brain regions primarily associated with the central actions of aldosterone on salt appetite and blood pressure regulation in the adult CNS. 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) is an NADP(H) dependant enzyme with bi-directional activity *in vitro*, but which acts predominantly as an 11β-reductase *in vivo* to catalyse the regeneration of active corticosterone. 11β-HSD1 is widely expressed in brain with high levels of mRNA expression, bioactivity and immunoreactivity found in hippocampus, cerebellum and cortex (Moisan *et al.*, 1990a,b; Lakshmi *et al.*, 1991; Sakai 1992), with lower levels of activity and immunoreactivity demonstrated in the brainstem (Moisan *et al.*, 1990a,b; Lakshmi *et al.*, 1991).

In this study, we investigated the presence of 11β-HSD1 activity in the MVN and vestibular-related areas of the cerebellum, in normal rats and in rats after vestibular deafferentation. The levels of 11β-HSD1 activity were determined in the vestibulocerebellum (nodulus and uvula, NU), the flocculus/paraflocculus (F/PF) complex and the MVN in normal rats, sham-operated rats, and rats that had compensated for 4 h after UL. We investigated the hypothesis that changes in 11β-HSD1 activity may contribute to the early stage of post-lesional plasticity in ipsi-lesional MVN neurons, by regulating the exposure of the MVN and cerebellum to glucocorticoids, during the time period when the increase in intrinsic excitability of the MVN neurons is taking place.

METHODS

Male Sprague–Dawley rats (80–120 g) underwent either sham operation ($n = 16$ rats) or UL ($n = 16$ rats) under avertin anaesthesia (300 mg/kg tribromoethanol, i.p.), as described previously (Cameron and Dutia, 1997; Yamanaka *et al.*, 2000). Animal care and experimental procedures were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986.

In brief, UL was carried out by opening the horizontal semicircular canal and the vestibule of the left inner ear using a 0.7 mm drill. The endolymph and the contents of the vestibule were aspirated, and the vestibule was rinsed with 100% ethanol. In sham-operated rats the horizontal canal was not opened and the inner ear was undamaged. The rats recovered in their home cages for 4 h post-UL, after which they were re-anaesthetised with halothane and decapitated by guillotine. The brain was quickly removed into ice-cold artificial cerebrospinal fluid (aCSF; composition in (mM): 124 NaCl, 5 KCl, 1.2 KH_2PO_4, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 26 $NaHCO_3$ and 10 D-glucose, equilibrated with 95% O_2/5% CO_2). The left and right F/PF complexes were dissected and immediately frozen on dry ice. The cerebellar hemispheres were removed and the caudal most folia containing the NU were dissected and frozen. The brainstem was cemented with the fourth ventricle uppermost to the stage of a Vibroslice, and 800 μm thick horizontal slices of the dorsal brainstem containing the MVN were cut. Each slice was separated along the midline, the left and right MVN were isolated by trimming and frozen. Dissected samples were stored at −72°C until use.

After homogenisation, total protein concentration was estimated colorimetrically (BioRad protein assay kit, Hemel Hampstead, UK). Tissue samples were diluted to a final assay concentration of 0.2 mg/ml protein in 8% glycerol, 150 mm NaCl, 1 mm EDTA, 50 mm Tris. Protein, 0.2 mg/ml, was incubated at 37°C with 10 nm ^{3}H-corticosterone and 400 mm of the 11β-HSD1 specific co-factor NADP, in a total volume of 250 μl. 11β-dehydrogenase activity was quantified in this assay as a measure of enzyme activity, since 11β-reductase is unstable in homogenates (Seckl, 1997). After incubation the reaction was stopped by the addition of ethyl acetate and steroids separated and assayed by thin layer chromatography (TLC). Conversion of corticosterone to 11-dehydrocorticosterone was quantified by densitometry of the resulting bands, and differences in activity were tested for statistical significance using one-way ANOVA (Low *et al.*, 1994; Jamieson *et al.*, 1997).

RESULTS

Figure 1 shows the results of an assay of 11β-HSD1 activity in MVN, F/PF and NU tissue pooled from four normal rats. The samples were incubated in parallel for 90 min, and each sample was assayed in duplicate. The values shown in Fig. 1 are thus the mean of two parallel, repeated assays for each tissue, carried out under identical conditions. High levels of 11β-HSD1 activity were observed in the F/PF and the NU, while 11β-HSD1 activity in the MVN was five fold lower. This finding is in line with previous reports showing high levels of 11β-HSD1 immunoreactivity, mRNA levels and bioactivity in cerebellum, and lower levels in the brainstem (Moisan *et al.*, 1990a; Lakshmi *et al.*, 1991). In addition, this result shows that high levels of 11β-HSD1 activity are found in the F/PF and NU, which are cerebellar areas intimately

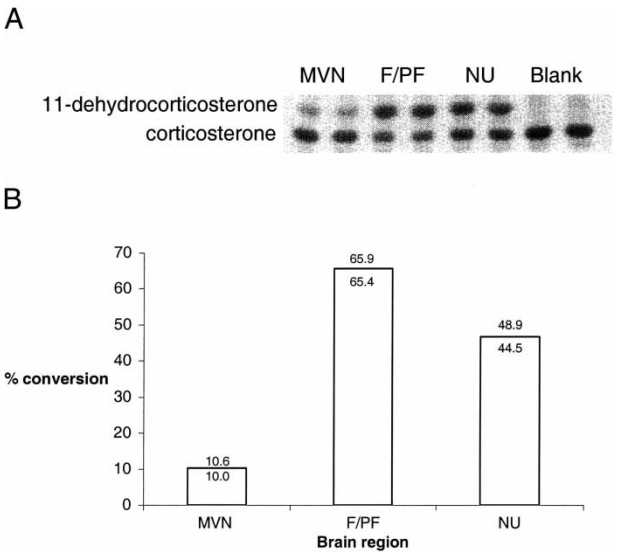

FIGURE 1 (A) Representative chromatogram showing conversion of ^{3}H-corticosterone to 11-dehydrocorticosterone in duplicate samples from MVN, F/PF complex and NU, and in a blank control which did not contain any brain homogenate. (B) 11β-HSD1 activity levels (expressed as % conversion of corticosterone to 11-dehydrocorticosterone) in MVN, F/PF and NU from normal rats ($n = 4$). Columns indicate means of assays repeated in duplicate, with the duplicate value points indicated above and below the mean line.

involved with the control of vestibular reflexes and eye movements.

Figure 2 shows 11β-HSD1 activity in MVN and F/PF tissue samples taken from normal rats, sham-operated rats and rats that had undergone VC for 4 h after UL.

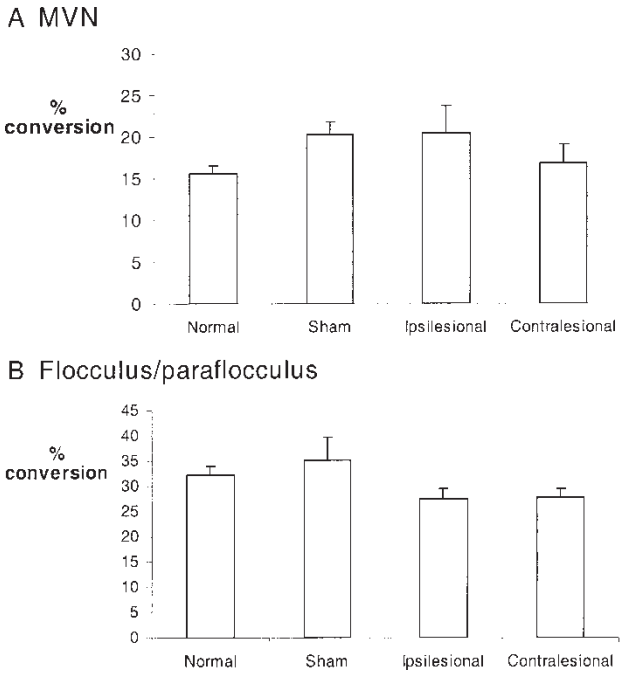

FIGURE 2 11β-HSD1 activity in ipsi- and contra-lesional MVN (A), and ipsi- and contra-lesional F/PF (B), from normal rats, sham-operated rats and rats that underwent VC for 4 h post-UL. In (A) and (B), columns indicate mean 11β-HSD1 activity ± SEM of four assays carried out in parallel under identical conditions, each assay consisting of tissue pooled from four rats.

Each column represents the average 11β-HSD1 activity ± SEM of four assays, each assay consisting of tissue pooled from four rats. Incubation times for each tissue type were adjusted taking into account the levels of activity demonstrated in the initial assay (Fig. 1), to maximise conversion of corticosterone without the reaction reaching saturation. Thus, F/PF samples were incubated for 40 min, and MVN samples for 330 min. There were no significant differences in the levels of 11β-HSD1 activity either between normal, sham and post-UL tissues, or between the ipsi- and contra-lesional MVN or F/PF after UL. In addition, there was no statistically significant difference between 11-β-HSD activity levels in NU tissue from normal, sham-operated and 4 h post-UL rats (data not shown).

DISCUSSION

VC is a multi-factorial process; rapidly developing compensatory mechanisms may be quite distinct from the longer term molecular and neuronal remodelling needed to sustain VC (Darlington and Smith, 2000). On a cellular level, there is evidence for plastic changes in immediate early gene and $GABA_A\alpha1$ receptor subunit expression, and glutamate release in vestibular related brain regions within 3–6 h after UL (Cirelli *et al.*, 1996; Kitahara *et al.*, 1997; Horii *et al.*, 2003; Inoue *et al.*, 2003). Electrophysiological studies have identified potential cellular mechanisms of plasticity in MVN neurons, which may play an important early role in the restoration of their resting activity after deafferentation. Within 4 h after UL in the rat, there is a significant down-regulation of the functional efficacy of inhibitory $GABA_A$ and $GABA_B$ receptors, and an up-regulation of the electrophysiological excitability, of the deafferented ipsi-lesional MVN cells (Cameron and Dutia, 1997; Yamanaka *et al.*, 2000; Johnston *et al.*, 2002). In addition, inhibitory glycine receptor efficacy is also down-regulated in the ipsi-lesional MVN cells (Vibert *et al.*, 2000). The changes in GABA receptor efficacy and intrinsic excitability are first observed at 4 h post-UL, and GR activation is required over this time for their manifestation (Cameron and Dutia, 1997; Guilding and Dutia, unpublished observations). However, the mechanisms by which GR activation facilitates these cellular changes in MVN neurons are unknown.

This study is the first to specifically investigate 11β-HSD1 activity in central vestibulooculomotor centres of the brainstem and cerebellum. The results demonstrate the presence of 11β-HSD1 activity in the MVN, F/PF and NU, and suggest that this modulatory enzyme is likely to be involved in regulating the exposure of these centres to circulating glucocorticoids. The high levels of 11β-HSD1 activity in F/PF and NU are of particular interest. These areas are likely to be rendered highly responsive to glucocorticoids, through the local amplification of active steroid concentrations by 11β-HSD1 activity. Indeed,

we have recently shown that the flocculus is an important site of genomically mediated glucocorticoid action in the initial stages of VC after UL, where glucocorticoids may facilitate synaptic plasticity in the cerebellar cortex (Johnston *et al.*, 2002). The functional significance of the lower level of 11β-HSD1 activity in the MVN remains to be established.

11β-HSD1 activity has been shown to be modulated by a number of hormones in peripheral tissues, and by glucocorticoids in peripheral and brain tissues (Seckl, 1997). Adrenalectomy in rats significantly reduces 11β-HSD1 mRNA expression in the hippocampus and 11β-HSD1 activity in the hippocampus, cerebellum and cortex at 10 days, but not at 1 day after adrenalectomy. These decreased 11β-HSD1 mRNA and activity levels are reversed, to levels above those found in sham-operated rats, by administration of the glucocorticoid agonist dexamethasone (Low *et al.*, 1994). Chronic (15 days) arthritic stress has also been shown to increase hippocampal 11β-HSD1 activity (Low *et al.*, 1994). By contrast, in the tree shrew chronic psychosocial stress (28 days) was found to attenuate hippocampal 11β-HSD1 activity (Jamieson *et al.*, 1997). Thus, induction of 11β-HSD1 in the brain during acute stress may help amplify intracellular glucocorticoid actions, whereas chronic attenuation of the enzyme activity may represent a homeostatic mechanism to ameliorate the otherwise excessive metabolic effects of glucocorticoid excess.

In this study, we hypothesised that changes in 11β-HSD1 activity may contribute to the early stage of post-lesional plasticity in MVN neurons, by regulating the exposure of the MVN and cerebellum to glucocorticoids, during the 4 h post-UL period when the changes in MVN neuronal properties are taking place. The results show that the levels of 11β-HSD1 activity in the vestibulocerebellum and MVN remain unchanged at the 4-h time-point after UL. They, therefore, disprove our hypothesis that changes in enzyme modulatory activity may occur in association with the initial UL-induced changes in the properties of the ipsi-lesional MVN neurons. The present study does not, however, address the possibility that changes in 11β-HSD1 activity may occur at later times after UL, or in other vestibular nuclei such as the lateral vestibular nucleus or its related cerebellar areas, during the overall process of VC.

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