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Molecular Brain Research 27 (1994) 174–178

MOLECULAR BRAIN RESEARCH

Increased glucocorticoid receptor gene expression in the rat hippocampus following combined serotonergic and medial septal cholinergic lesions

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Accepted 19 July 1994

Abstract

Glucocorticoid excess is associated with hippocampal neuronal dysfunction and loss, mainly affecting CA1. Degeneration of both cholinergic and serotonergic (5-HT) hippocampal afferents is prominent in aged rats and Alzheimer's disease. Lesions of these individual pathways alter hippocampal expression of mineralocorticoid (MR) and glucocorticoid (GR) receptor mRNAs; both transcripts are increased by cholinergic lesions, but markedly *decreased* by serotonergic denervation. In the present study we found that combined medial septal cholinergic and central 5-HT lesions increase hippocampal GR mRNA expression, specifically in CA1 and CA2 subfields, whereas MR mRNA expression was similar to controls. Thus the effects of the cholinergic lesion, at least upon GR gene expression, appear to predominate while the effects of the lesions upon MR gene expression were additive. Increased hippocampal GR gene expression per neuron may increase hippocampal neuronal vulnerability with age or disease.

Keywords: Glucocorticoid receptor; Mineralocorticoid receptor; Gene expression; Aging; Hippocampus; In situ hybridization

Hippocampal glucocorticoid (GR) and mineralocorticoid (MR) receptor gene expression are influenced little, at least in the longer term, by glucocorticoids themselves [10], whereas neurotransmitters released from hippocampal afferents provide potent control of receptor gene expression [25,30,31,32]. The hippocampus receives major 5-HT and cholinergic inputs from the midbrain raphe nuclei and the medial septal area, respectively [1,6]. Dysfunction of both pathways are features of affective and age-related cognitive disorders [3,29]. These conditions are also associated with glucocorticoid hypersecretion and attenuated feedback control of the hypothalamic-pituitary-adrenal (HPA) axis [15], functions mediated, in part, by hippocampal GR and MR. Recent studies suggest that combined cholinergic/5-HT lesions potentiate the effects of each lesion alone upon hippocampal electrical activity and spatial memory in the rat [21,28]. Moreover, hippocampal cognitive deficits, such as those seen in Alzheimer's disease and with age, are thought to result from the combined loss of different modulatory neurotransmitters, in particular cholinergic and 5-HT innervations [2,3]. We have previously shown that medial septal cholinergic lesions increase hippocampal MR and GR mRNA expression [30], and have suggested that this might increase GR-mediated neuron-endangering effects in aged animals and man, particularly in the face of glucocorticoid hypersecretion [17,23,26]. By contrast, 5-HT lesions alone markedly decrease hippocampal MR and GR mRNA expression [25,31]. The present study was therefore designed to examine the consequence of combined lesions of hippocampal 5-HT and cholinergic innervations upon corticosteroid receptor gene expression, using in situ hybridization histochemistry, and to compare these effects with those induced by the individual lesions, as published previously.

All surgery on male Wistar rats (250–300 g; n = 5–7/group) was done in a stereotaxtic apparatus under halothane (3.5%) anaesthesia. For 5-HT lesions, 5,7-di-

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SSDI 0169-328X(94)00158-8

hydroxytryptamine (5,7-DHT) (Sigma, UK) was given as a single injection (200 μg/20 μl) into the right lateral ventricle (i.c.v.); 3 min were allowed for diffusion before syringe retraction. Stereotaxic co-ordinates, with bregma as reference, were: A = 0.0; L = 1.4; H = 4.5 (mm). Controls received vehicle alone i.c.v. (saline containing 0.2% ascorbic acid, 20 μl). Rats were injected i.p. with desipramine (Sigma, 25 mg/kg) 30 min prior to 5,7-DHT injection to protect the noradrenergic system.

Medial septal lesions were made electrolytically 6 days after 5,7-DHT lesions using a Grass DC constant current lesion maker (DC-LM5) and 100 μm diameter stainless steel electrodes (current intensity 15 mA for 13 s using the 0.5 mm uninsulated tip of a stereotaxically-implanted electrode). Stereotaxic co-ordinates, with bregma as reference, were A 0.6; L 0.0; H 5.9 (mm). Control rats (vehicle i.c.v.) had electrodes implanted in the medial septum, but no current was applied.

After 14 days animals were killed (09.00–11.00 h), the brains removed and immediately frozen on dry ice. Coronal brain sections (10 μm) at the level of the dorsal hippocampus were cut, thaw-mounted onto gelatine-subbed, poly-L-lysine-coated slides and stored at −85°C. One representative slide from each rat was stained for acetylcholinesterase (AChE) according to the method of Koelle and Friedenwald [14] to check the location and extent of the medial septal lesion. 5,7-DHT lesions were verified by estimating the loss of 5-HT uptake sites on hippocampal terminals using [^{3}H]paroxetine autoradiography according to the protocol of DeSouza and Kuyatt [4]. Analysis of [^{3}H]paroxetine binding autoradiograms was performed using computer-based image analysis (Quantimet 970; Cambridge Instruments, UK). For each hippocampal subfield of interest, optical density measurements were made bilaterally from six consecutive autoradiographic images, and local tissue tracer concentrations were derived by comparison of the mean optical density of the area of interest with the optical densities of precalibrated tritium-containing standards. Specific binding was determined by subtraction of non-specific binding images (incubated in the presence of citalopram) from the total binding (incubation with [^{3}H]paroxetine alone). Data were assessed using Student's *t*-test for grouped data.

In situ hybridisation histochemistry was carried out as described previously [25]. Brain sections were post-fixed in 4% paraformaldehyde, washed and hybridised in situ using [^{35}S]UTP-labelled cRNA probes transcribed in vitro from rat cDNA clones encoding MR and GR (513 bp and 674 bp, respectively). Denatured probes were added to hybridization buffer (10×10^6 cpm/ml) and 200 μl aliquots were pipetted onto slides and hybridized overnight. Sections were treated with RNase A (30 mg/ml, 45 min at 37°C), washed to a final stringency of 0.1 × SSC at 60°C, dehydrated, dipped in photographic emulsion and exposed for 21 days before being developed and counterstained with 1% pyronine. Silver grains were counted using computerised image analysis (Seescan plc, Cambridge, UK) over individual hippocampal neurons (15–20 cells per subfield for each animal) within a fixed circular frame, except in the dentate gyrus where it was difficult to determine cell boundaries; here the fixed circular frame covered approximately one neuron with parts of adjacent neurons. Results were calculated as mean grain counts/neuron for each region after subtraction of background (counted over areas of white matter). Data were assessed by ANOVA followed by post-hoc Scheffé test. Values are presented as mean ± S.E.M.

All septal cholinergic lesions were complete as assessed by the absence of acetylcholinesterase (AChE) staining in the hippocampus compared to sham-lesioned controls (Fig. 1). Intracerebral administration of

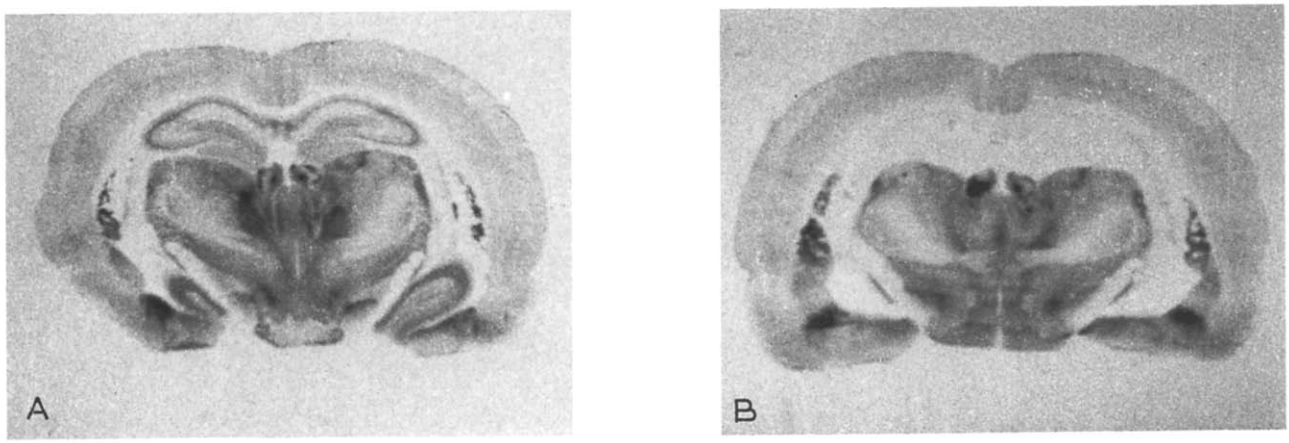

Fig. 1. Coronal brain sections from (A) control and (B) combined cholinergic/5-HT-lesioned animals, stained for acetylcholinesterase. Note the absence of staining in the hippocampus with lesioning.

Table 1
[^{3}H]Paroxetine binding in hippocampal subregions following combined medial septal and 5,7-DHT lesions

	n	CA1	CA3	DG
Sham (5,7-DHT + septal)	4	72 ± 2	202 ± 17	42 ± 3
5,7-DHT + septal	4	6 ± 4 *	13 ± 5 *	7 ± 3 *

Data are expressed as fmol bound/mg protein (mean ± S.E.M.). DG, dentate gyrus.
* $P < 0.01$.

5,7-DHT significantly decreased [^{3}H]paroxetine binding to 5-HT nerve terminals in the hippocampus (> 90% decrease, see Table 1). Combined lesions did not cause any obvious neuron loss or damage in the hippocampus, as assessed by histological inspection.

In sham-lesioned controls, GR mRNA expression was higher in the dentate gyrus, CA1 and CA2 than in CA3 and CA4, whereas MR mRNA expression was similar in all hippocampal subregions, in agreement with previous reports [9,25]. Combined septal and 5-HT lesions had no significant effect on MR mRNA expression in any hippocampal subregion (Fig. 2). In contrast, GR mRNA expression was significantly increased (Fig. 2) by approximately 22% in CA1 ($P < 0.05$, e.g. Fig. 3) and CA2 ($P < 0.01$); levels in CA3, CA4 and the dentate gyrus remained unchanged. Neuronal GR mRNA expression in the overlying parietal cortex was unaltered.

Medial septal lesions increase both MR and GR mRNA expression by 20–30% in specific subregions of the hippocampus [30]. Central 5-HT lesions, in contrast, attenuate both MR and GR mRNA expression by up to 50% in most hippocampal subfields [25,31]. In the present study, combined cholinergic and 5-HT lesions resulted in unaltered hippocampal MR mRNA expression. Both lesions were complete, as indicated by the absence of AChE staining and the near abolition of [^{3}H]paroxetine binding in the hippocampus. The overall absence of effect of combined cholinergic/5-HT lesions suggests an additive action upon hippocampal

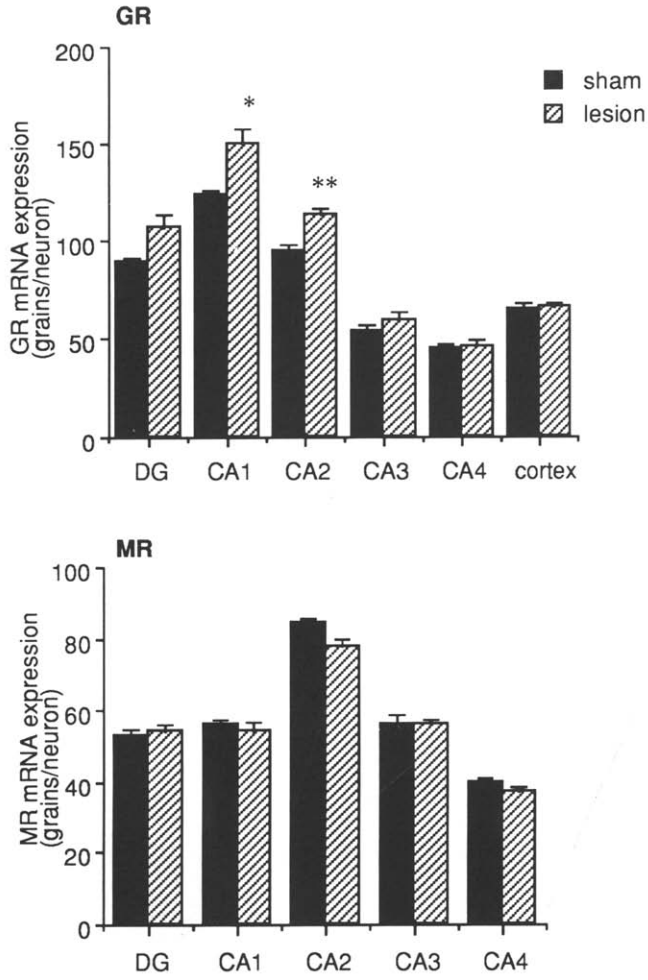

Fig. 2. MR and GR mRNA expression in hippocampal subregions from control ($n = 5$) and combined cholinergic/5-HT-lesioned animals ($n = 7$). DG, dentate gyrus; CA1–CA4, subregions of Ammon's horn. * $P < 0.05$, ** $P < 0.01$ compared to control.

MR gene expression and support suggestions of a functional interaction between cholinergic and 5-HT systems innervating the hippocampus. Consistent with this, anatomical studies have shown that 5-HT raphe

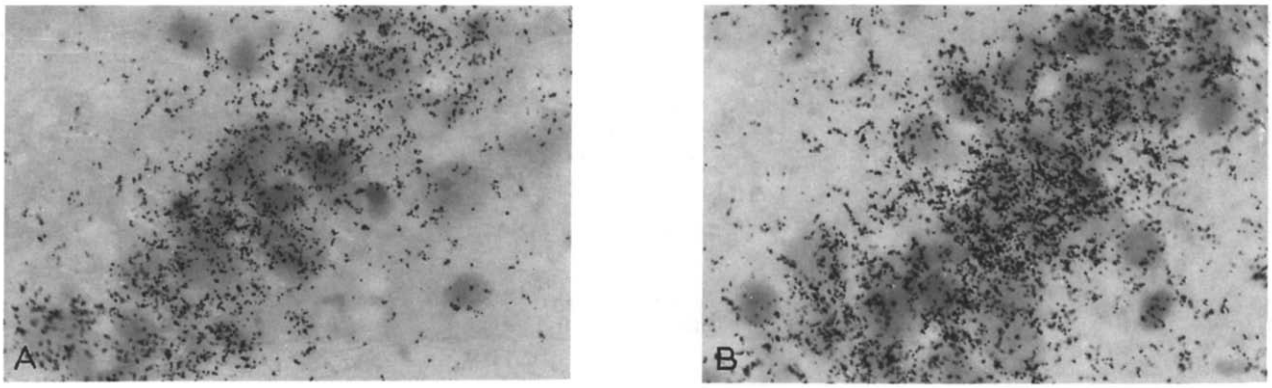

Fig. 3. Bright-field photomicrograph (×450) showing GR mRNA expression in CA1 pyramidal cells from a sham-lesioned control (A) and a combined cholinergic/5-HT-lesioned rat (B). Silver grains, which appear black under bright-field, represent GR mRNA expression. Note increased GR mRNA expression in combined cholinergic/5-HT-lesioned CA1 neurons.

neurons innervate both the area containing septal cholinergic neurons and the hippocampus [27].

In contrast, hippocampal GR mRNA expression was significantly increased from control following combined lesions, to a similar extent as cholinergic lesions alone, presumably reflecting the dominance of the cholinergic lesion, at least in CA1 and CA2. Indeed, cells in CA1 may be more sensitive to acetylcholine than those in CA3–4, perhaps due to the denser cholinergic innervation [8]. The mechanism responsible for increased GR gene expression in CA1–2 is unclear, but appears to be specific to the hippocampus, since expression in the overlying cortex was not affected. This increase is also unlikely to be the result of altered circulating corticosterone levels, since even major manipulations of corticosteroids have little or no long-term effect on MR and GR mRNA expression [9,10]. Intriguingly, chronic environmental enrichment also increases GR gene expression in CA1–2, an effect which is closely associated anatomically with altered expression of transcription factors (NGFI-A and NGFI-B) putatively regulated by nerve-growth factor (NGF) [19]. Hippocampal NGF is crucial for the maintenance of function of the cholinergic innervation [5]. Moreover, hippocampal NGF levels are increased by both environmental enrichment and septo-hippocampal cholinergic lesioning [7,18]. Thus cholinergic denervation may induce hippocampal NGF and its related transcription factors, which may act directly on the GR promoter, with specificity for CA1–2, perhaps due to interactions with other transcription factors in this region, induced/repressed by the coincident monoaminergic deficit.

Chronically elevated glucocorticoid levels are associated with hippocampal neuronal loss, and aged rats are more sensitive to these actions [13]. Moreover, CA1 is the prime target for hippocampal neuronal loss in rats following hypoxia-ischaemia [20] and with age-related glucocorticoid hypersecretion [12] and in humans with Alzheimer's disease [11]. We and others have suggested that loss of plasticity of receptor expression may explain the particular vulnerability of the aging hippocampus to glucocorticoids [16,26]. In the face of combined hippocampal cholinergic/5-HT denervation, elevated hippocampal GR mRNA expression, and presumably although not inevitably GR binding sites, might increase vulnerability to the neuron-jeopardising actions of glucocorticoids which are mediated via GR [22]. In support of this idea, corticosterone administration for 3 months results in loss of hippocampal neurons preferentially in Ammon's horn, an area with neurons bearing the highest GR density [24]. Whether combined cholinergic/5-HT lesions indeed increase hippocampal neuronal vulnerability to glucocorticoids remains to be determined.

We thank June Noble for excellent technical assistance and Drs. R. Evans, J. Arriza and R. Miesfeld for kindly supplying the cDNA clones used in this study. This research was supported by a Wellcome Trust/Royal Society of Edinburgh Senior Clinical Research Fellowship (J.R.S.).

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