Corticosterone modifies muscarinic receptor immunoreactivity in rat hippocampus


Department of Animal Physiology, Graduated School of Behavioural and Cognitive Neurosciences, University of Groningen, The Netherlands
Institute for Animal Science and Health (ID-DLO), Lelystad, The Netherlands

Received 3 February 1999; received in revised form 12 April 1999; accepted 14 April 1999

Abstract

In the present study we report the effect of corticosterone in the regulation of hippocampal muscarinic acetylcholine receptor immunoreactivity (mAChR-ir) expression in rats. Adrenalectomy (ADX) or a single injection of a mineralocorticoid antagonist RU-28318 (1.0 mg/100 g body weight (b.w.)) in adrenally intact rats 24 h prior to sacrifice revealed an increased mAChR-ir in hippocampal CA1 and CA3 areas. Corticosterone replacement (100 μg/100 g b.w.) prevented the increase in mAChR-ir of ADX animals. However, glucocorticoid receptor antagonist (RU38486) treatment in adrenally intact rats failed to affect the mAChR immunolabeling. These results point to a modulation of muscarinic receptors by corticosterone that is predominantly mediated by the mineralocorticoid receptor.

Glucocorticoid hormones, corticosterone (in rat) or cortisol (in human), are known to influence brain processes such as mood, behavior and memory [4,14]. These actions of circulating corticosteroid hormones are mediated via intracellular corticosteroid receptors. At present, two different corticosterone receptor types are known in the brain: the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) [4]. The high density of MRs and GRs in hippocampal neurons suggests that corticosteroids are important regulators of the function of this structure [4]. Several biochemical [2,9,18] and electrophysiological studies [12,13] have proposed a role for corticosterone in cholinergic neurotransmission in the hippocampus. The hippocampal formation and in particular the cholinergic septohippocampal system have been implicated in the neural substrate for spatial learning, predominantly through the action of acetylcholine on muscarinic acetylcholine receptors (mAChR) [10,19]. It has also been shown that altering levels of plasma corticosterone affects spatial learning [6,16].

Previous results from our laboratory revealed that successful training performance in the hole board spatial discrimination task causes an increase and subcellular redistribution of mAChR immunoreactivity (mAChR-ir) in hippocampal pyramidal cells [1,7,19,20]. In view of the putative effects of corticosteroids on cholinergic neurotransmission and spatial memory processes, we examined hippocampal mAChR expression in relation to experimental manipulations of corticosteroids and their receptors and studied the impact of adrenalectomy and differential MR and GR blockade in adrenally intact animals on mAChR-ir in the CA1, CA3 and the dentate gyrus (DG) regions.

The experiments were approved by the Committee on Animal Bio-Ethics of the University of Groningen. 42 male Wistar rats (290–340 g) were used, housed in groups of six animals per cage and kept on a 12:12 h light/dark cycle (lights on at 07:00 h) with food and water available ad libitum. The experiments were carried out between 09:00 and 12:00 h.

Six animals were sham-adrenalectomized (Sham) and 12 animals were bilaterally adrenalectomized (ADX) under ether anesthesia. Six animals of the latter group received a...
single subcutaneous injection (S.C.) of corticosterone in a dose of 100 μg/100 g body weight (cortADX) producing physiological serum levels of corticosterone for at least 24 h [5], whereas the other animals were injected (S.C.) with the vehicle control solution immediately after the surgical procedure. For antagonist treatment 24 intact rats were divided into four groups of six each: control (vehicle-treated), aMR (anti-mineralocorticoid treated), aGR (anti-glucocorticoid treated), and aMR/aGR (treated with both antagonists). The MR antagonist (RU28318; 3,3-oxo-7-propyl-17hydroxy-androstan-4-en-17yl-propionic acid-lactone) [17] and the GR antagonist (RU38486; 17β-hydroxy-11β-(4-dimethyl amino-phenyl)17α-(1-propynyl)estro-4,9-diene-3-one) [8,15] were kindly provided by Roussel-UCLAF, Romainville, France. Both steroids were®rst dissolved in ethanol and subsequently diluted in polyethylene glycol 400 (PEG; BDH, Poole, UK) until a ®nal ethanol concentration of 2%. The vehicle control contained the same PEG/ethanol concentration. RU28318 was injected (S.C.) with a dose of 1.0 mg/100 g body weight, whereas RU38486 was given in a dose of 2.5 mg/100 g body weight. Injections (0.2 ml) were given at 10:30 h. The effects of various treatments on mAChRs were studied 24 h later.

Prior to transcardial perfusion the rats were deeply anaesthetized with ether and perfused with 300 ml of a ®xative consisting of 3% paraformaldehyde, 0.05% glutaraldehyde and 0.2% picric acid in 0.1 M phosphate buffer (PB) at pH 7.4. Fixation was preceded by a prerinse with 50 ml saline solution. The brains were removed, stored overnight in 30% buffered sucrose at 4°C for cryoprotection, and coronally sectioned on a cryostat microtome at a thickness of 20 μm. Immediately before perfusion, 0.5 ml aterial blood was taken from the left atrium to determine plasma corticosterone levels. The blood samples were immediately transferred to centrifuge tubes containing 10 μl heparin solution (500 units/ml) and centrifuged for 20 min at 3500 × g. The supernatant was stored at −20°C for the corticosterone assay. Corticosterone was extracted from 75 μl plasma and determined by HPLC with UV detection at 254 nm according to Dawson et al. [3]. The absolute detection threshold for corticosterone in plasma was 8 ng/ml. The intra- and interassay coef®cients were 3 and 8%, respectively.

Muscarinic receptor protein immunocytochemistry using the mouse anti-mAChR IgM M35 (Chemunex, Paris, 1:200) was performed on free ®oating brain sections as previously reported [20]. The distribution of M35-immunoreactivity was quanti®ed by measuring the relative optical density (OD) by way of an image analysis system (Quantimet 600, Leica) as described in detail elsewhere [20]. For quanti®cation three adjacent sections per animal containing the hippocampus were analyzed bilaterally. The data were averaged and calculated per group. The OD of the ADX, cortADX, aMR, aGR, and aMR/aGR groups were compared with the OD of the control groups and statistically analyzed using the Kruskal–Wallis ANOVA followed by the Mann–Whitney U test. A probability level of P < 0.05 was taken as statistical signi®cance for all tests.

Plasma corticosterone levels (mean ± SEM) of Sham

---

Fig. 1. Representative photomicrographs of the hippocampal CA1 area immunostained to mAChRs of Sham (a), ADX (b), cortADX (c), aMR/aGR (d), aMR (e) and aGR (f) treated rats.
Changes in the level of mAChR-binding may further be interpreted in terms of changes in mAChR-mediated cholinergic neurotransmission. Recent electrophysiological studies have shown that corticosterone dose-dependently modulates responsiveness of CA1 pyramidal neurons to the cholinergic agonist carbachol in hippocampal slices from ADX rats [12,13]. Large carbachol responses were recorded in the presence of very low doses of corticosterone, which are possibly mediated by MRs. Moreover, moderate amounts of corticosterone significantly suppressed the depolarizing effects of carbachol. Although it should be noted that different designs and time schedules are used in the above mentioned studies, our findings and literature all point to an MR- rather than GR-mediated impact of corticosterone on the mChR.

Changes in the level of mAChR-ir alters the engagement of the hippocampal system in learning and memory. The present findings are in line with the autoradiographic results of Biegon et al. [2]. In the latter study rats were adrenalectomized, while half of the animals were supplied with a corticosterone pellet under the skin after which the brains were processed for mChR autoradiography. Binding of [3H]QNB in the dorsal hippocampus was reduced in adrenalectomized, corticosterone supplemented rats as compared with ADX animals. The changes in mAChR-binding may further be interpreted in terms of changes in mAChR-mediated cholinergic neurotransmission. Recent electrophysiological studies have shown that corticosterone dose-dependently modulates responsiveness of CA1 pyramidal neurons to the cholinergic agonist carbachol in hippocampal slices from ADX rats [12,13]. Large carbachol responses were recorded in the presence of very low doses of corticosterone, which are possibly mediated by MRs. Moreover, moderate amounts of corticosterone significantly suppressed the depolarizing effects of carbachol. Although it should be noted that different designs and time schedules are used in the above mentioned studies, our findings and literature all point to an MR- rather than GR-mediated impact of corticosterone on the mChR.

Changes in the level of mAChR-ir alters the engagement of the hippocampal system in learning and memory.

Fig. 2. Relative optical density of M35-ir in the rat hippocampal subfields of sham ADX, ADX, ADX animals treated with corticosterone and animals treated with mineralocorticoid receptor antagonist, glucocorticoid receptor antagonist, or a combination of both antagonists. M35-ir was significantly increased in the CA1 and CA3 pyramidal cell layer of ADX treated animals (A), and in the CA1, CA3 and DG of aMR-, aMR/aGR treated animals (B) when compared with controls. *Indicates significance level (P < 0.05). Scale bars, means ± SEM.

(n = 6), cortADX (n = 5), aMR/aGR (n = 5), aMR (n = 4), and aGR (n = 5) were 7.2 ± 3.1, 8.2 ± 1.9, 16.3 ± 2.5, 11.7 ± 3.5 and 6.2 ± 2.7 μg/dl, respectively. Plasma corticosterone levels showed the tendency to increase after aMR or aMR/aGR treatment, but data did not reach significance (P < 0.06). The corticosterone levels of all ADX animals (n = 6) were below the detection limit of 8 ng/ml.

mAChR-ir in the CA1 area is depicted in Fig. 1. mAChR-ir in sham-ADX (Fig. 1a) resembled that of behaviorally naive control animals [7,20]. The between-groups comparison indicated a significant treatment effect on mAChR-ir in CA1 and CA3 fields (P < 0.01; P < 0.04; Kruskal–Wallis test), respectively. Post-hoc testing revealed a significantly increased immunostaining of the ADX group in both cornu ammonis fields CA1 and CA3 (P < 0.05; Fig. 2A). This increase was the result of dense mAChR-ir in pyramidal cell bodies and apical dendrites (Fig. 1b). The ADX-induced increment, however, was reduced to control level with predominant immunopositive interneurons by application of corticosterone immediately after ADX (Figs. 1c and 2A). The labeling-intensity in the molecular layer of the DG was less pronounced and non-significantly affected by ADX or cort/ADX.

The effects of antagonist treatment on the changes in mAChR-ir in CA1, CA3, and DG molecular layer are shown in Figs. 1d–f and 2B. Kruskal–Wallis analysis of the data revealed a significant effect between controls and antagonist treated animals in these hippocampal regions (P < 0.01; P < 0.03; P < 0.04), respectively. Administration of the aMR or a combination of both aMR and aGR profoundly (P < 0.05) enhanced mChR immunostaining in CA1 (Figs. 1e,f and 2B), CA3 (Fig. 2B), and in the molecular layer of the DG (Fig. 2B).

In contrast to the aMR- or aMR/aGR treated animals the GR blockade alone induced no significant increase in the mAChR labeling-intensity (Figs. 1f and 2B).

In general, the present results indicate that 24 h after adrenalectomy or selective blockade of mineralocorticoid receptors, mAChR-ir in the hippocampal formation of the rat is strikingly enhanced. MR blockade and adrenalectomy resulted in a similar increase in mAChR-ir, indicating that corticosterone modulates mChRs through an MR- rather than a GR-mediated pathway. This conclusion is further supported by the finding that aGR treatment did not affect mAChR-ir. Furthermore, a combined aMR/aGR treatment resulted in the same enhancement as aMR alone. Since the occupation of MRs is already high at low levels (i.e. baseline) of corticosterone the interaction between corticosterone and hippocampal mChRs is rather tonic than phasic (i.e. reactive) of character.

Changes in the level of mAChR-ir alters the engagement of the hippocampal system in learning and memory.
processes. Alterations in mAChR-sensitivity is one way to regulate the impact of cholinergic neurotransmission. Based on the observations on enhanced hippocampal mAChR-ir after training in a spatial learning paradigm [1,7,20], increased levels of mAChR-ir are most likely due to an altered functional state of the muscarinic receptor (e.g. internalized and/or phosphorylated) that renders it less sensitive to, and hence inhibits the impact of acetylcholine [19]. It has been postulated that acetylcholine is a modulator switching the hippocampus from recall to learning mode upon release [11]. Acetylcholine exerts this shift partly by activating postsynaptic mAChRs on hippocampal principal cells. High levels of mAChR-ir (visualized by using the monoclonal antibody M35) reflects a hippocampal system relatively insensitive for acetylcholine, which corresponds to a recall mode rather than learning mode [19]. Enhanced mAChR-ir after ADX, therefore, points to hippocampal engagement in recall rather than acquisition [19], which may contribute to reduced spatial learning performance as seen after ADX [16].

We thank Roussel–Uclaf (Romainville, France) for the use of RU28318 and RU38486. We acknowledge J. Gast for excellent technical assistance and D. Visser for preparing the figures.