Corticosterone strongly increases the affinity of dorsal raphe 5-HT$_{1A}$ receptors

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The effects of corticosterone (10 mg/kg, s.c., 6 h) on dorsal raphe 5-HT$_{1A}$ autoreceptors have been studied in adrenalectomized rats with or without porcine galanin modulation. Adrenalectomy diminishes 5-HT$_{1A}$ autoreceptors affinity. Corticosterone increases 5-HT$_{1A}$ autoreceptor agonist affinity (+90%, p < 0.001) in adrenalectomized rats. Galanin (10 nM) increases dorsal raphe 5-HT$_{1A}$ autoreceptor density (+65%, p < 0.05) and its Kd value (+248%, p < 0.05) only in adrenalectomized rats treated with corticosterone. Dorsal raphe glucocorticoid receptors activation by corticosterone may therefore lead to an increased signalling of 5-HT$_{1A}$ autoreceptors that may become counteracted by galanin receptor activation. Glucocorticoids, by enhancing dorsal raphe 5-HT$_{1A}$ autoreceptor function, may therefore cause reduced 5-HT neuronal activity and thus lead to a depressive state. NeuroReport 15:1457–1459 © 2004 Lippincott Williams & Wilkins.

Key words: Corticosterone; Dorsal raphe; Galanin; 5-HT$_{1A}$

INTRODUCTION

Increased glucocorticoid levels and a hyperfunction of the HPA axis activity, and enhanced dorsal raphe (DR) 5-HT$_{1A}$ autoreceptor function and blunted postsynaptic 5-HT$_{1A}$ receptor signalling have been reported in depressed patients [1–6]. Acute hydrocortisone diminishes the postsynaptic 5-HT$_{1A}$ receptor function in healthy human volunteers [7]. Intracerebral galanin reduces DR activity inter alia via interaction with 5-HT$_{1A}$ autoreceptors [8–10] contributing to behavioural signs of depression in animals and an up-regulation of DR galanin receptors has in fact been reported in a genetic model of depression [11]. The adrenal steroid effects were therefore studied on the rat DR 5-HT$_{1A}$ receptor binding characteristics with and without galanin modulation.

MATERIALS AND METHODS

The experimental procedures followed the provisions and general recommendations of the European Communities Council Directive (86/609/EEC), and have been approved by the local ethical committee (Stockholms Norra Försöksd-jurs Etsika nämnd). Adult male Sprague–Dawley rats ($n=12$) were bilaterally adrenalectomized (ADX) under halothane anaesthesia. The sham group ($n=6$) underwent the same surgical procedure but only with exposure of the adrenal glands. All surgical procedures and hormone injections were performed between 07:00 and 09:00 h. After ADX (24 h) the rats were injected with a single dose of corticosterone (10 mg/ml/kg, s.c.). This corticosterone dose activates both glucocorticoid receptors and mineralocorticoid receptors. The sham group was injected with propylene glycol (hormone vehicle). The animals were decapitated 6 h after the injections and coronal sections of the brains were obtained (bregma –7.3 mm, 14 mm). Cresyl violet staining of tissue sections was used for neuroanatomical identification [12]. The 5-HT$_{1A}$ receptors were characterized by quantitative receptor autoradiography with the 5-HT$_{1A}$ selective agonist $[^3]$H-8OH-DPAT (0.24–26 nM, sp. act. 224 Ci/mmol) with and without porcine galanin (1–29) 10 nM as described earlier [10]. The $[^3]$H$8$OH-DPAT binding sites of the DR were quantitatively analysed from the receptor autoradiograms by a computer-assisted image analyzer. A standard square of 0.08 mm$^2$ placed over the region of the DR was used to assess the autoradiograms. Saturation analysis was performed (Graph-Pad PRISMA software) to determine the affinity (Kd) and the maximal receptor density ($B_{max}$) values. Trunk blood samples were collected in EDTA-containing tubes for analyses of serum corticosterone levels by radioimmunoassay (RIA) using $[^3]$Hcorticosterone (corticosterone assay detection limit = 5.7 ng/ml) as described earlier [13]. No measurable levels of serum corticosterone were found in ADX rats. Serum corticosterone levels were 244 ±30 ng/ml in the sham group and 48 ±12 ng/ml in the ADX+corticosterone-treated group, in agreement with earlier findings at 8 h [14].
One-way ANOVA repeated measures followed by Scheffé post-test was performed in the statistical analysis using the Statistical Package for Social Sciences 9.0.

RESULTS

Effects of adrenalectomy: ADX produced a marked increase in the $K_d$ value of the DR 8OH-DPAT binding sites (ADX group 14.6 $\pm$ 2nM vs sham group 7.7 $\pm$ 0.6 nM, $p<0.05$; Fig. 1). Galanin (10 nM), at a concentration known to reduce the affinity of the postjunctional 5-HT$_1A$ receptors [10,15] did not alter the $K_d$ and the $B_{max}$ values of 8OH-DPAT binding sites of the DR in either ADX or the sham groups (Fig. 2).

Effects of a single injection of corticosterone in adrenalectomized rats: Corticosterone treatment (10 mg/kg, 6 h), increased the affinity (1.3 $\pm$ 0.3 nM, decrease in $K_d$ value by 87.4%, $p<0.001$) with no change in the $B_{max}$ values of the DR 8OH-DPAT binding sites of the ADX animals (ADX group 68 $\pm$ 11 fmol/mg protein, corticosterone 70 $\pm$ 8 fmol/mg protein, sham group 46 $\pm$ 6 fmol/mg protein; Fig. 1). Galanin (10 nM) after corticosterone treatment increased the $B_{max}$ (64%) and specially the $K_d$ (248%) values of 8OH-DPAT binding sites in the DR of the ADX animals with respect to the basal values ($p<0.05$; Fig. 2).

DISCUSSION

Adrenalectomy markedly reduces the affinity of DR 5-HT$_{1A}$ agonist binding sites as previously indicated [16,17]. Corticosterone reverses this effect into one of a marked increase in the affinity of the DR 5-HT$_{1A}$ agonist binding sites, which becomes significant also vs the sham-operated animals. Corticosterone treatment via activation of the glucocorticoid receptors in the DR [18] can markedly enhance the affinity and the signalling of the DR 5-HT$_{1A}$ autoreceptors [10,15,19]. This change may be related to corticosterone-induced changes in gene expression of 5-HT$_{1A}$ receptor interacting proteins that by protein-protein interactions with 5-HT$_{1A}$ receptors can change the conformational state of this receptor producing a marked increase in the affinity of the agonist site of the dorsal raphe 5-HT$_{1A}$ receptors (for comparison see [20]). This 5-HT$_{1A}$ autoreceptor function enhancement may lead to reduction of the firing rate in DR 5-HT nerve cells diminishing the 5-HT releasing activity of the 5-HT nerve terminals in the tel-diencephalic regions. In this way it is
possible to see how glucocorticoids may produce depression and is consistent with the serotonergic hypothesis of depression [21].

After corticosterone treatment an increase of the Bmax and especially of the Kd values occurred after in vitro galanin modulation, using a concentration known to modulate 5-HT1A receptors in the forebrain [15,19,22]. Previous studies have reported that an important reduction in 5-HT1A receptor agonist affinity occurs after galanin modulation [10,15,19,23]. The corticosterone-induced increase in 5-HT1A autoreceptor affinity may lead to an increase in galanin receptor affinity in the DR via the enhancing 5-HT1A-galanin receptor interaction as has also been reported in studies on hippocampal 5-HT release in vivo [24]. The post- and pre-junctional level galanin/5-HT1A receptor reciprocal antagonistic interactions leading to a reduction of the affinity of 5-HT1A agonist binding sites [15,19] may therefore become enhanced after the corticosterone treatment. Such an increase of the 5-HT1A-galanin receptor interaction can explain the selective reduction of 5-HT1A receptor affinity by galanin in the DR of the corticosterone-treated ADX animals vs ADX animals. The intra-membrane antagonistic galanin receptor-5-HT1A receptor interaction can act as a compensatory inhibitory mechanism to reduce the affinity of the 5-HT1A autoreceptors thus aiming to counteract the corticosterone-induced sensitization of these receptors. The DR galanin receptors can also signal by themselves and hyperpolarize the dorsal raphe cells via opening K+ channels [25] reducing the firing rate of DR 5-HT cells with inhibition of 5-HT neurotransmission in the terminal areas, contributing to the production of a depressive state.

CONCLUSION
Corticosterone increases the dorsal raphe 5-HT1A autoreceptor affinity of adrenalectomized rats probably leading to a reduced 5-HT neuronal activity. The corticosterone-induced increase in the signalling of the 5-HT1A autoreceptors may lead to an increase in the 5-HT1A/galanin receptor interaction resulting in an increase of galanin receptor signalling which also may reduce the firing rate of the dorsal raphe. The possible reduction of 5-HT neurotransmission by corticosterone may contribute to a depressive state.

REFERENCES