

Effect of subchronic corticosterone administration on α_2 -adrenoceptor functionality in rat brain: an in vivo and in vitro study

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Abstract

Rationale Noradrenergic system plays a critical role in the hypothalamic-pituitary-adrenal (HPA) axis regulation and the stress response. A dysregulated HPA axis may be indicative of an increased biological vulnerability for depression. In addition, a variety of studies have focused on specific alterations of α_2 -adrenoceptors as a mechanism involved in the pathogenesis of mood disorders and antidepressant response. **Objectives** This study aimed to evaluate the effect of subchronic corticosterone administration on rat brain α_2 -adrenoceptor functionality by in vitro [³⁵S]GTP γ S binding stimulation assays and in vivo dual-probe microdialysis determination of extracellular noradrenaline concentrations. **Results** Implantation of a time release corticosterone pellet during 14 days induced sustained changes in endocrine function. However, there were no differences in α_2 -adrenoceptor agonist UK14304-induced stimulation of [³⁵S]GTP γ S binding in prefrontal cortex (PFC) between corticosterone-treated and control rats. In the same way, the in vivo evaluation of α_2 -adrenoceptor-mediated noradrenaline release responses to the α_2 -adrenoceptor agonist clonidine local administration into the locus coeruleus (LC), and the PFC did not show differences between the groups. **Conclusions** The present results show that subchronic corticosterone administration does not induce changes on

functionality of α_2 -adrenoceptors neither in the LC nor in noradrenergic cortical terminal areas.

Keywords Adrenoceptor · Corticosterone · HPA · Mood disorders · Depression

Introduction

Noradrenergic system and hypothalamic-pituitary-adrenal (HPA) axis are both activated in response to stress. On the one hand, HPA axis activation leads to corticosterone release which promotes the mobilization of resources, among others, peripheral monoamine release or central nervous system regulation. It is released to compensate for adverse effects of stressful events and become especially relevant when stress is maintained for a long time. On the other hand, stress results in an increased synthesis, release and turnover of noradrenaline (NA) in several brain regions involved in the stress response, including cerebral cortex, hippocampus, hypothalamus and amygdala (Stanford 1995). The main source of noradrenergic innervation in the central nervous system (CNS) remains in the *locus coeruleus*. Furthermore, inhibitory α_2 -adrenoceptors modulate noradrenergic neurone activity and NA release both in locus coeruleus (LC) and noradrenergic terminal areas (Mateo and Meana 1999). Alterations in these α_2 -adrenoceptors have been associated to psychiatric disorders, such as anxiety or depression, and also to the effect of antidepressants drugs. In this sense, an upregulation of α_2 -adrenoceptors has been reported in the brain of depressed subjects and/or suicide victims (Callado et al. 1998; De Paermentier et al. 1997; Valdizan et al. 2010), as well as in platelets of depressed untreated patients (Garcia-Sevilla et al. 2004). In contrast,

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decreased α_2 -adrenoceptor binding sites have been described in platelets from patients with anxiety disorders with respect to the control subjects (Bremner et al. 1996). Moreover, chronic administration of antidepressants reduces the activity of α_2 -adrenoceptors in rat brain (Invernizzi et al. 2001; Mateo et al. 2001; Parini et al. 2005) and in human platelets (Garcia-Sevilla et al. 1990; Garcia-Sevilla et al. 2004). However, the role of noradrenergic system in chronic stress conditions remains poorly understood. The notion that chronic stress exposure could be related to affective disorders is supported by findings of altered HPA axis function in depressed and anxious subjects. Thus, altered 24-h urinary cortisol excretion, blunted adenocorticotrophin hormone (ACTH), responses to exogenous corticotrophin releasing factor (CRF) administration, and increased concentration of CRF in the cerebrospinal fluid (CSF) has been reported in patients suffering from depression (Charmandari et al. 2005).

Numerous animal models have been developed for the purpose of studying mood and anxiety disorders. In this sense, exogenous corticosterone administration has been described as a useful method to study the relationship between stress, glucocorticoids, and depression/anxiety. Exogenous corticosterone administration in animals provides a valid and reliable paradigm to study the possible influence of stress system dysregulation on the aetiology, development, and treatment of anxiety and depression (Sterner and Kalynchuk 2010). In this context, the aim of the present study was to evaluate the effect of subchronic corticosterone administration for 14 days in rat brain α_2 -adrenoceptor in vitro and in vivo functionality.

Materials and methods

Animals and treatments

Male Sprague-Dawley rats (256 ± 4 g; $n = 33$) were obtained from Harlan Interfauna Ibérica, S.A. (Barcelona, Spain) and were treated subchronically with corticosterone or vehicle. Commercially available corticosterone pellets (Innovative Research of America, USA) designed to release 56 mg of corticosterone during 14 days ($16 \text{ mg kg}^{-1} \text{ day}^{-1}$) were used. Control pellets (Innovative Research of America, USA) were exactly the same formulation but in the absence of corticosterone. Subcutaneous pellets were implanted in the back of the neck by surgical procedure. Animal care and all experimental protocols were performed in agreement with the European Ethical Standards (European Union Directive 2010/63/EU) and translation into Spanish legislation

(RD 53/2013) and approved by the UPV/EHU Ethical Board for Animal Welfare (CEEAA).

Plasma corticosterone determination

Fourteen days after surgery, animals were killed by decapitation and trunk blood was collected in EDTA tubes. Blood was centrifuged at 3000g for 20 min at 4 °C, and plasma was removed and stored at -80 °C until use. Plasma samples were analysed using a commercially available radioimmunoassay kit for corticosterone (sensitivity 5.7 ng mL^{-1} ; Coat-a-count® rat corticosterone, DPS DIPESA S.A., Madrid, Spain). The adrenal glands were also dissected and weighed.

[^{35}S]GTP γ S binding assays in membrane preparation

Rat brain cortex samples were immediately dissected after animal decapitation and stored at -80 °C until assay was performed. Membranes from whole cortex were prepared as previously described (Gonzalez-Maeso et al. 2000). The α_2 -adrenoceptor-mediated functional G-protein activity was assessed by [^{35}S]GTP γ S binding assay as previously described (Gonzalez-Maeso et al. 2000) with slight modifications to adapt the incubation conditions to 96-well microplates. The α_2 -adrenoceptor full agonist UK14304 (10^{-9} to $10^{-3} \text{ mol l}^{-1}$) was used to determine receptor-stimulated [^{35}S]GTP γ S binding concentration-response curves. The specific binding was determined as the difference between total and nonspecific binding defined as the remaining [^{35}S]GTP γ S binding in the presence of $10 \text{ }\mu\text{M}$ GTP γ S.

Surgery, microdialysis procedure, drug administration and chromatography

At day 13 after pellet implantation, animals were anaesthetised and two microdialysis probes were implanted in the proximity of the right LC and in the ipsilateral PFC as previously described (Ortega et al. 2012). In these conditions, in vitro recovery for NA was in the 10–15 % range. After 24 h for recovery, experiments were performed. In order to evaluate α_2 -adrenoceptor functionality, the agonist clonidine was dissolved in artificial CSF (0.1 – $100 \text{ }\mu\text{M}$, four concentrations) and administered locally by reverse dialysis in the LC or PFC as previously described (Fernandez-Pastor et al. 2013). Clonidine was selected as α_2 -adrenoceptor agonist because of the existence of previous validated data of its activity in vivo (Mateo and Meana 1999; Mateo et al. 2001). NA concentrations were measured immediately after collection of the samples by HPLC coupled to an electrochemical detector as previously described (Ortega et al. 2012).

Data analyses

Data from [³⁵S]GTPγS binding stimulations are expressed as percentage of maximal stimulation (E_{\max}) over specific basal binding. Nonlinear regression analysis was performed on individual concentration-response curves of the agonist. The concentration of drug that elicited half-maximal effect (EC_{50}) was obtained and normalized as $-\log EC_{50}$ value before statistical analysis. All data are expressed as mean values \pm SEM. Differences of [³⁵S]GTPγS binding parameters between different experimental groups were assessed by unpaired Student's *t* test. The level of significance was chosen at $p < 0.05$.

In microdialysis experiments, statistical analyses were performed on normalized values as percentages of the basal concentrations. One-way ANOVA followed by Dunnett's post-hoc test was used to assess the effect of the drug over time. The effect of clonidine on corticosterone-treated animals was compared with the effect on the control group by two-way ANOVA of repeated measures followed by Bonferroni's *post-hoc* test. In these analyses, all the experimental points, including basal values, were considered. F values were expressed as F_{tr} (treatment; between the groups), F_t (time; within groups) or F_i (treatment \times time; interaction). Maximal effects (E_{\max}) were expressed as percentage over basal values. The area under the curve (AUC) values of each curve was also calculated by the addition of the percent change from basal value. Differences of AUC between different groups were assessed by unpaired Student's *t* test. Results are expressed as mean values \pm SEM. All statistical procedures were performed using GraphPad Prism™ (GraphPad Software, San Diego, CA, USA).

Results

Physiological parameters

At day 14, after pellet implantation, plasma corticosterone concentration was higher in the group treated subchronically with corticosterone (141 ± 19 ng ml⁻¹) than in the control group (72 ± 18 ng ml⁻¹) ($p = 0.02$) (Fig. 1a). Body weight was also measured and the weight gain was lower (2 ± 4 g) than in control group (34 ± 7 g) ($p < 0.001$) (Fig. 1b). Thus, adrenal gland weight was corrected for body weight before comparison between the groups, and as expected, a significant reduction of adrenal gland size in corticosterone-treated group was found ($p < 0.001$) (Fig. 1c).

[³⁵S]GTPγS binding assays

The [³⁵S]GTPγS basal binding was similar in the group treated subchronically with corticosterone (1075 ± 135 fmol mg prot.⁻¹,

$n = 12$) and in the control group (813 ± 165 fmol mg prot.⁻¹, $n = 8$). The α_2 -adrenoceptor agonist UK14304 stimulated [³⁵S]GTPγS binding in a concentration-dependent manner (10^{-9} – 10^{-3} M, ten concentrations) in both groups (Fig. 2a). The potency of UK14304 to stimulate [³⁵S]GTPγS binding in corticosterone-treated group ($-\log EC_{50} = 7.0 \pm 0.3$) was not different to that in the control group ($-\log EC_{50} = 6.7 \pm 0.3$). UK14304-induced maximal stimulation of [³⁵S]GTPγS binding did not differ between the groups (corticosterone $E_{\max} = 14 \pm 2$ % vs control $E_{\max} = 12 \pm 2$ %) either.

Microdialysis

Basal extracellular NA concentrations in LC and PFC

Basal extracellular NA concentrations were obtained as average of three consecutive basal samples per animal. There were no statistical changes of basal extracellular NA concentration in LC in corticosterone-treated group (2.74 ± 0.49 nM; $n = 36$) when compared to the control group (2.53 ± 0.93 nM; $n = 23$). In the same way, no alterations of basal extracellular NA concentrations in PFC were observed in corticosterone-treated group (3.30 ± 0.74 nM; $n = 36$) in comparison with controls (3.60 ± 0.70 nM; $n = 28$).

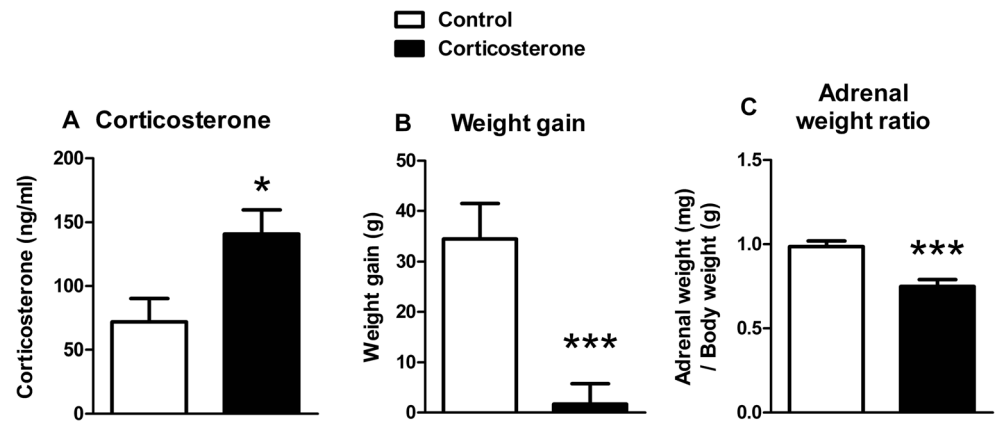
Effect of local administration of clonidine into PFC on extracellular NA concentration evaluated in PFC

Local administration of the α_2 -adrenoceptor agonist clonidine (0.1–100 μ M, four concentrations) into the PFC induced a significant decrease of extracellular NA concentration in PFC of corticosterone-treated ($E_{\max} = -56 \pm 11$ %, $p < 0.0001$) ($F[10,66] = 12.79$; $p < 0.0001$; $n = 7$) and control ($E_{\max} = -48 \pm 16$ %, $p < 0.001$) ($F[10,55] = 4.55$; $p = 0.0001$; $n = 6$) groups (Fig. 2b). There were not significant differences in the effect induced by clonidine between the groups ($F_{tr}[1,11] = 1.50$; $p = 0.25$; $F_t[10,110] = 14.10$; $p < 0.0001$; $F_i[10,110] = 1.18$; $p = 0.31$). The analysis of the AUC values revealed a decrease (14 %) in the corticosterone-treated group that did not reach statistical significance ($t = 1.17$; $p = 0.27$).

Effect of local administration of clonidine into LC on extracellular NA concentrations evaluated in LC and PFC

Local administration of clonidine (0.1–100 μ M, four concentrations) into the LC significantly decreased extracellular NA concentration in LC in a concentration-dependent manner (Fig. 3a) in control ($E_{\max} = -41 \pm 1$ %, $p < 0.001$) ($F[10,66] = 14.57$; $p < 0.0001$; $n = 7$) and in corticosterone-treated ($E_{\max} = -42 \pm 7$ %, $p < 0.001$) ($F[10,88] = 12.93$; $p < 0.0001$; $n = 9$) groups. There were not significant

Fig. 1 **a** Plasma corticosterone concentration, **b** body weight gain and **c** adrenal: body weight ratio obtained for each experimental condition. Rats received corticosterone ($16 \text{ mg kg}^{-1} \text{ day}^{-1}$) or vehicle during 14 days from subcutaneous pellet. Data are shown as mean \pm SEM ($n = 8$ for each group; $*p < 0.05$; $***p < 0.0001$ compared with control)



differences in the effect induced by local administration of clonidine in LC between experimental groups ($F_{t[1,14]} = 0.08$; $p = 0.77$; $F_{t[10,140]} = 24.17$; $p < 0.0001$;

$F_{i[10,140]} = 1.42$; $p = 0.18$). As expected, the percentage of decrease measured as AUC was not different between the groups ($t = 0.11$; $p = 0.91$).

Local administration of clonidine into the LC also induced a significant decrease in the extracellular NA concentration in PFC both in control ($E_{\text{max}} = -46 \pm 12 \%$, $p < 0.001$) ($F[10,77] = 14.26$; $p < 0.0001$; $n = 8$) and in the corticosterone-treated groups ($E_{\text{max}} = -49 \pm 16 \%$, $p < 0.001$) ($F[10,88] = 13.93$; $p < 0.0001$; $n = 9$) (Fig. 3b). The observed effect was not different between the groups

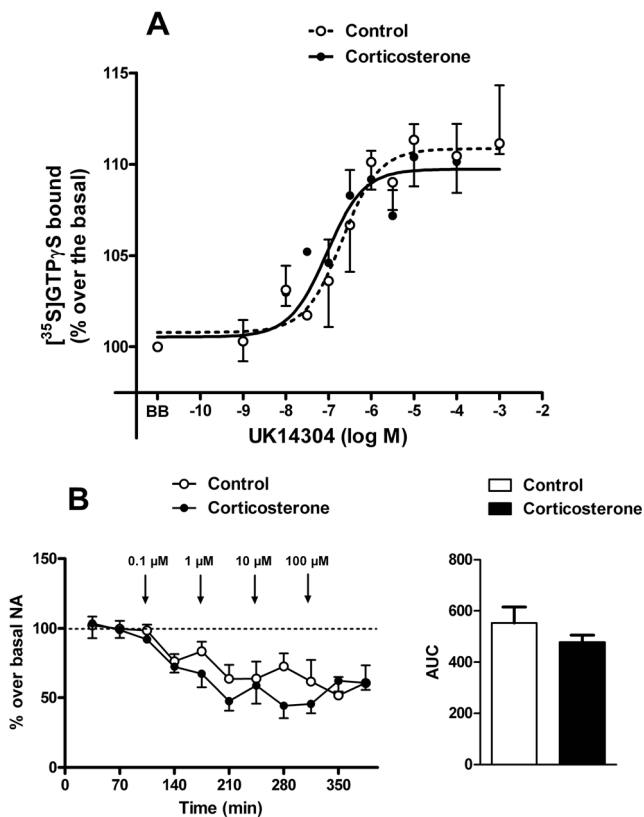


Fig. 2 **a** Concentration-response curves of [^{35}S]GTP γ S binding stimulation by α_2 -adrenoceptor agonist UK14304 (10^{-9} to 10^{-3} M, ten concentrations) in rat brain cortex membrane homogenates of the control and corticosterone-treated animals. Points represent mean \pm SEM values from eight to 12 experiments carried out in triplicates and are expressed as percentages over basal binding (BB) values. **b** Effect of local administration of the α_2 -adrenoceptor agonist clonidine (0.1–100 μM) in PFC by reverse dialysis over extracellular NA concentrations in PFC. Rats received corticosterone ($16 \text{ mg kg}^{-1} \text{ day}^{-1}$) or vehicle along 14 days from subcutaneous pellet. Data are shown as mean \pm SEM values from seven experiments and are expressed as percentages of the corresponding basal values. Bar graphs represent mean \pm SEM area under the curve expressed in arbitrary units

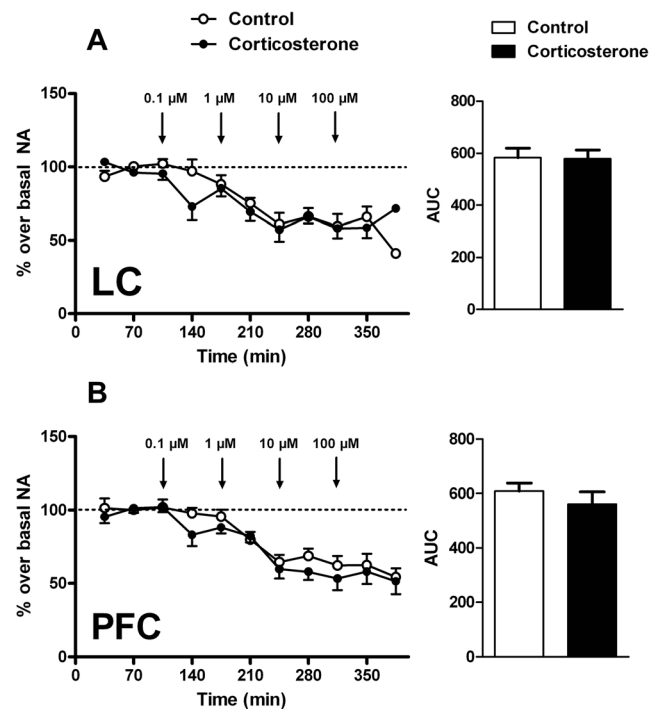


Fig. 3 Effect of local administration of the α_2 -adrenoceptor agonist clonidine (0.1–100 μM) into the LC by reverse dialysis on extracellular NA concentrations in LC (**a**) and PFC (**b**). Data are shown as mean \pm SEM values from 7 to 9 experiments and are expressed as percentages of the corresponding basal values. Rats received corticosterone ($16 \text{ mg kg}^{-1} \text{ day}^{-1}$) or vehicle along 14 days from subcutaneous pellet. Bar graphs represent mean \pm SEM area under the curve (AUC) in arbitrary units

($F_{tr}[1,15] = 1.34$; $p = 0.26$; $F_i[10,150] = 27.23$; $p < 0.0001$; $F_i[10,150] = 0.50$; $p = 0.88$). When the effect induced by clonidine was measured as AUC, there were not significant differences between the groups ($t = 0.88$; $p = 0.39$).

Discussion

A wide variety of studies have linked dysfunctions in central noradrenergic system and HPA axis with stress and psychiatric disorders, particularly anxiety and depression. In the present study, we observed that subchronic corticosterone administration for 14 days, even though producing profound changes in the HPA axis related physiological parameters, did not induce alterations in the functionality of brain α_2 -adrenoceptors, analysed by both in vitro and in vivo methods.

Several animal models have been developed for the purpose of studying depressive symptomatology and response to antidepressant drugs. Animal models of chronic mild stress have been described as useful paradigms for studying the neurobiological bases of mood disorders (Hill et al. 2012). In that respect, chronic stress exposure induces functional changes of α_2 -adrenoceptors in LC neurones. These changes are region-specific and time-dependent leading to some contradictory results in the literature. A single cortisol injection, which induces similar effects to those triggered by stress, downregulates α_2 -adrenoceptor density in several brain regions of adult male tree shrews, whereas the oral administration of cortisol for 5 days induces regional receptor protein upregulation (Flugge 1999). In the same way, chronic psychosocial stress in male tree shrews during 10 days downregulates brain α_2 -adrenoceptors but an upregulation after 28 days is also observed (Flugge 1996). On the other hand, chronic exposure to cold during 14 days, another paradigm of stress, induces the desensitization of α_2 -adrenoceptors in LC (Jedema et al. 2008).

The possible role of glucocorticoids in the stress-induced abnormalities on noradrenergic system remains unclear. In this sense, exogenous corticosterone administration in animals has also been described as a useful model of mood disorders (Sterner and Kalynchuk 2010). Thus, functional desensitization of somatodendritic 5-HT_{1A} autoreceptors has been described as a consequence of subchronic corticosterone administration (Leitch et al. 2003) with a similar protocol to the one applied in the present work. The 5-HT_{1A} receptors are the main inhibitory serotonin receptors that regulate the firing of serotonergic raphe neurones, affecting serotonin levels at projection sites throughout the brain. Thus, these receptors play a similar role for serotonergic system as α_2 -adrenoceptors do in the noradrenergic function.

In the present work, α_2 -adrenoceptor functionality was studied in rats treated with time release corticosterone pellets during 14 days. This treatment induces a flattened diurnal

blood corticosterone rhythm without raising the total daily amount of circulating corticosterone. As described in previous works (Gartside et al. 2003; Leitch et al. 2003), corticosterone-induced HPA axis dysfunction was observed in physiological parameters such as adrenal and body weight and plasma corticosterone concentration.

Interestingly, basal concentrations of NA did not differ between corticosterone-treated and control rats, indicating that corticosterone treatment does not affect the basal release of NA from noradrenergic neurones in LC or PFC. The α_2 -adrenoceptors located in these areas regulate noradrenergic neurone activity and NA release via inhibitory G-proteins ($G_{i/o}$). Results obtained in [³⁵S]GTP γ S binding assays showed that in corticosterone-treated rats, the functional coupling to G-proteins of α_2 -adrenoceptors located in PFC is not altered. However, α_2 -adrenoceptors functionality evaluated by in vitro [³⁵S]GTP γ S binding assays does not discriminate between auto and heteroreceptors. Therefore, microdialysis was used to evaluate the functionality of the subpopulation of α_2 -adrenoceptor located on noradrenergic neurones acting as inhibitory feedback input that controls NA release to the synaptic cleft in both LC and PFC areas. In order to evaluate this α_2 -adrenoceptor sensitivity in vivo, we used the well-characterized α_2 -adrenoceptors selective agonist clonidine (Mateo and Meana 1999). It has been previously described that local administration of clonidine in the PFC, a noradrenergic terminal area, induces a decrease of local NA release by acting on presynaptic α_2 -adrenoceptors (Mateo et al. 2001). Our results confirm that clonidine-induced NA decrease in PFC was similar in both experimental groups and demonstrate that no differences in the functionality of these receptors are induced by the present paradigm of corticosterone treatment.

The firing activity of LC noradrenergic neurones is controlled by somatodendritic α_2 -adrenoceptors located in this area exerting a tonic inhibitory effect on NA release by noradrenergic terminal areas (Fernandez-Pastor and Meana 2002). Therefore, NA concentration in PFC is tightly controlled by α_2 -adrenoceptors-mediated inhibitory modulation of LC firing rate (Mateo et al. 1998). In the present study, we measured NA concentrations in the PFC after local administration of clonidine into the LC in order to evaluate the role of this somatodendritic α_2 -adrenoceptor subpopulation expressed in the LC area. There were no differences of clonidine response when NA concentration was measured in PFC. In the other hand, it has been described another subpopulation of α_2 -adrenoceptors located in LC that regulates somatodendritic NA release in this area (Callado and Stamford 1999; Fernandez-Pastor and Meana 2002). The evaluation of functionality of this α_2 -adrenoceptor population in the LC did not show changes associated to the corticosterone treatment.

Although there are evidences of corticosterone-induced effects on rat physiology and in contrast to previously observed

evidences in somatodendritic 5-HT_{1A} receptors, α_2 -adrenoceptors show resistance in the present paradigm. However, this fact does not exclude a putative role of corticosterone as inductor of α_2 -adrenoceptor alterations on aetiology of mood disorders after longer modulations of corticosterone levels. Here, we cannot completely exclude the risk of making a type II or β error (failing to reject the false null hypothesis), because of the size of the sample. Indeed, taking into account that clonidine administration induced a 14-% reduction in NA release in PFC of corticosterone-treated rats (Fig. 2), the sample size needed to ensure a 0.80 statistical power would be as high as 40 rats per group. Therefore, potential differences, if existing, would be of low biological relevance.

Despite there is not any alteration in α_2 -adrenoceptor functionality, we cannot preclude definitive alterations in noradrenergic system. In this regard, here α_2 -adrenoceptor activity has been evaluated in LC and PFC, but we cannot discard changes in other noradrenergic projection areas such as hippocampus. On the other hand, we have measured extracellular NA concentration in basal conditions and, therefore, the possibility of an altered noradrenergic system in response to acute stress conditions in corticosterone-treated animals cannot be discarded.

It is important to remark that alterations induced by stressors on α_2 -adrenoceptor functionality seem to be time-dependent. In fact, the density of α_2 -adrenoceptors and probably also the sensitivity of the receptor system are dependent on the duration of the stress period. Similarly, the treatment length and/or dose could also be important in the pharmacological response to corticosterone (Sterner and Kalynchuk 2010). In this line, there are evidences suggesting that long-term treatments are necessary to alter adrenoceptor functionality, as it has been demonstrated for administration of different antidepressant treatments (Invernizzi et al. 2001; Mateo et al. 2001). In this context, evaluation of α_2 -adrenoceptors functionality on a longer corticosterone treatment paradigm could be of interest.

The present data demonstrate that corticosterone administration during 14 days by time release subcutaneous pellet did not induce alterations on α_2 -adrenoceptor functionality in noradrenergic neurones in the CNS. Larger treatments would be necessary to confirm or discard that α_2 -adrenoceptors are sensitive to long-term corticosterone effects.

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Compliance with ethical standards Animal care and all experimental protocols were performed in agreement with the European Ethical Standards (European Union Directive 2010/63/EU) and translation into Spanish legislation (RD 53/2013) and approved by the UPV/EHU Ethical Board for Animal Welfare (CEEA)

Conflict of interest The authors declare that they have no conflict of interest.

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