Acta histochemica **112** (2010) 147—160

Available online at www.sciencedirect.com
ScienceDirect

acta histochemica
www.elsevier.de/acthis

Single-dose and chronic corticosterone treatment alters c-Fos or FosB immunoreactivity in the rat cerebral cortex

Réka Szakács[a], Ildikó Fazekas[b], András Mihály[b,*], Beáta Krisztin-Péva[b], Anna Juhász[a], Zoltán Janka[a]

[a]*Department of Psychiatry, Faculty of Medicine, University of Szeged, Szeged, Hungary*
[b]*Department of Anatomy, Faculty of Medicine, University of Szeged, PO Box 427, H-6701 Szeged, Hungary*

Received 12 July 2008; received in revised form 8 October 2008; accepted 14 October 2008

KEYWORDS
Corticosterone;
c-Fos;
FosB;
Immunohisto-chemistry;
Cerebral cortex;
Rat

Summary

The aim of this study was to examine the effects of single-dose and chronic corticosterone treatment on the inducible transcription factor c-Fos and FosB, and thereby to estimate the effects of high-doses of corticosterone on calcium-dependent neuronal responses in the rat cerebral cortex. At the same time we investigated the distribution of interneurons containing calretinin (CR), vasoactive intestinal polypeptide (VIP) and neuropeptide Y (NPY) in chronically treated animals in order to collect data on the involvement of inhibitory neurons in this process. Adult male rats were injected subcutaneously with 10 mg corticosterone, whereas controls received the vehicle (sesame oil). The animals were fixed by transcardial perfusion 12 and 24 h following single corticosterone injection, and the brains were processed for c-Fos and FosB immunohistochemistry. To investigate the effects of repeated corticosterone administration, rats were daily treated with the same amount of corticosterone (10 mg/animal, subcutaneously) for 21 days. Controls were injected with vehicle. At the end of the experiment, the rats were perfused and immunohistochemistry was used to detect the presence of the FosB protein, CR, VIP and NPY. Quantitative evaluation of immunolabelled cells was performed in the neocortex and the hippocampus. The number of immunoreactive nuclei per unit area was used as a quantitative measure of the effects of corticosterone. It was found that a single-dose administration of corticosterone resulted in a significant, time-dependent increase of c-Fos protein immunoreactivity in the granule cell layer of the dentate gyrus, as well as in regions CA1 and CA3 of the hippocampus 12 and 24 h post-injection with respect to control animals. Significant enhancement of c-Fos immunoreactivity was also observed in the neocortex at 12 and 24 h post-injection.

*Corresponding author. Tel.: 36 62 545 665; fax: +36 62 545 707.
E-mail address: mihaly@anatomy.szote.u-szeged.hu (A. Mihály).

0065-1281/$ - see front matter
doi:10.1016/j.acthis.2008.10.001

Single-dose treatment did not significantly alter FosB immunolabelling. Repeated administration of corticosterone produced a complex pattern of changes in FosB immunolabelling: significant increase in FosB immunoreactivity was detected in the granule cell layer of the dentate gyrus, with no significant changes in the CA1 and CA3 layers of the hippocampus and in the neocortex. However, a significant decrease of FosB induction in the neocortex was observed in chronically treated rats in comparison to single-dose injected animals (12 h before immunohistochemistry). Analysis of immunohistochemical detection of interneuronal markers revealed a significant reduction of the CR immunolabelling in the CA3 area of the hippocampus. No changes in VIP or NPY immunoreactivity were found in the Ammon's horn 3 weeks following daily corticosterone treatment. NPY immunoreactivity was significantly attenuated in the neocortex.

The present data suggest that single-dose corticosterone treatment increases immunoreactivity of c-Fos protein in a time-dependent manner, 12 and 24 h post-injection in the rat hippocampus and the neocortex, whereas chronic corticosterone treatment influences FosB immunoreactivity, primarily in the dentate gyrus. Chronic corticosterone administration seems to affect CR levels in the CA3 area of the hippocampus.

Introduction

Several lines of evidence indicate that glucocorticoids influence activity of brain structures and that excess glucocorticoid may have deleterious consequences in the central nervous system, predominantly in the hippocampus (Sapolsky et al., 1995; Joëls, 2001). Chronic exposure to excess glucocorticoid could endanger hippocampal cell function and morphology, leading to decreased neurogenesis and increased dendritic atrophy in the hippocampus and prefrontal cortex, which might be involved in memory deficits and ensuing psychopathology (Gould and Tanapat, 1999; Sapolsky, 2000; Wellmann, 2001). Apart from the chronic effects of glucocorticoids, they may have acute, short-term neuronal effects, the molecular mechanisms of which still remain to be clarified (Haller et al., 1998). The mechanisms of action of glucocorticoids in the central nervous system comprise both genomic and non-genomic effects (Wolkowitz et al., 2001; Joëls, 2001). Most of the effects of glucocorticoids are mediated by modulation of gene transcription, through interaction with corticosteroid receptors: the activated receptors translocate to the nucleus and affect gene expression either directly by binding to DNA as homodimers or indirectly, interacting with other transcription factors (Ayroldi et al., 2002). The hippocampus is a primary target site for glucocorticoids, the receptors for glucocorticoids being densely located in the Ammon's horn, as well as in the septum and amygdala, regions of the brain thought to be intimately involved in behavior, learning and memory function (McEwen et al., 1986; De Kloet, 1991; Wolkowitz et al., 2001). The prefrontal cortex is similarly a behaviorally relevant target for glucocorticoids since stress alters prefrontal cortical functions and glucocorticoid receptors (Rajkowska, 2000; Wolkowitz et al., 2001).

Corticosterone, the endogenous glucocorticoid of most rodents, binds to central corticosteroid receptors, the mineralocorticoid and glucocorticoid receptors, which are co-expressed in the neurons of limbic regions, such as the CA1 pyramidal and dentate granule cells of the hippocampal formation (Joëls, 2001). Low levels of corticosterone are sufficient to activate mineralocorticoid receptors (which are mostly restricted to limbic brain regions), whereas activation of hippocampal glucocorticoid receptors only occurs at high corticosterone levels, as seen after stressful events or following peripheral corticosterone administration (Reul et al., 1987; Joëls, 2001). Potential targets for corticosteroid actions that could alter neuronal membrane properties are the voltage-gated ion channels (Kerr et al., 1992; Karst et al., 1994) and neurotransmitter-mediated receptors, including ligand-gated ion channels and G-protein-coupled receptors (Joëls, 2001; Czyrak et al., 2002; Wiegert et al., 2005). Exposure to high levels of corticosterone has been reported to increase Ca^{2+} influx into hippocampal cells through voltage-gated Ca^{2+} channels by glucocorticoid receptor activation (Kerr et al., 1992), resulting in enhanced cell firing accommodation. However, prolonged exposure to high concentrations of corticosterone and glucocorticoid receptor activation may lead to excessive and uncontrolled Ca^{2+} influx with

enhanced vulnerability and cell death (Joëls, 2001). In addition to the marked sensitivity of the voltage-gated Ca^{2+} conductances to corticosteroids, high concentrations of the hormone modulate glutamate and/or GABA-mediated fast synaptic transmission, including the development of long-term potentiation (LTP) related to *N*-methyl-D-aspartate (NMDA)-receptor-mediated Ca^{2+} influx in the CA1 region of the hippocampus within 1 h (Vidal et al., 1986; Kawato et al., 2001; Shibuya et al., 2003). High concentrations of corticosterone administered acutely (within 30 min) decrease NMDA-receptor-mediated Ca^{2+} elevation in the CA1 region in hippocampal slices (Sato et al., 2004). Nevertheless, these rapid modulatory effects of corticosteroids are not only fast in onset, but often also reversible within minutes (for a review, see: Joëls, 2001), revealing the possibility that corticosteroids may drive, apart from their genomic effects, acute non-genomic pathways (Joëls, 2001; Sato et al., 2004).

Members of the activator protein 1 (AP-1) family, c-Fos and FosB belong to the inducible proto-oncogenes, which exert various regulatory actions in the cell nucleus, including late-response gene transcription that may be critical for neuronal adaptive responses (Morgan and Curran, 1995; Herdegen and Leah, 1998; Greenberg and Ziff, 2001). Among the transcription factors encoded by these immediate early genes (IEGs) are members of the Fos family of proteins, such as Fos, FosB, Fra-1 and Fra-2, all possessing leucine zippers. These IEGs are induced by different stimuli, including membrane depolarisation and Ca^{2+} influx. Activation of c-Fos transcription requires Ca^{2+} influx, mediated mainly by neurotransmitters acting on ionotropic receptors, such as NMDA and AMPA types of glutamate receptors and through voltage-sensitive Ca^{2+}-channels (Greenberg and Ziff, 2001). Fos immunohistochemistry has widely been used in neuropharmacological investigations to determine neuronal activation patterns. Accordingly, previous studies from our laboratory indicated that immunohistochemical detection of Fos protein serves as a marker of neuronal activation in forebrain structures (Mihály et al., 2001; Szakács et al., 2003; Fazekas et al., 2006). In the experiments reported here, we investigated the changes in c-Fos and FosB immunolabelling following a single injection of corticosterone and we tested the alterations of FosB immunoreactivity after chronic corticosterone treatment in order to estimate the effects of high-doses of corticosterone on calcium-dependent neuronal responses. Several investigations have revealed the fundamental role of inhibitory neurons in the control of (calcium-dependent) synaptic mechanisms, synaptic plasticity and network activity in the hippocampus (Acsády et al., 1996; Freund and Buzsáki, 1996). We therefore used immunolabelling to identify calcium-binding protein, calretinin (CR)-containing interneurons, as well as interneurons containing neuropeptides, such as vasoactive intestinal polypeptide (VIP) and neuropeptide Y (NPY), which label specific populations of interneurons.

Materials and methods

Animals and treatments

Adult male Wistar rats weighing 200–220 g were used in the study. The animals were housed in standard laboratory conditions at constant temperature and humidity, under a 12 h light–dark cycle. The rats had free access to water and food. The experiments were conducted in accordance with prevailing laws and ethical considerations. Written permission was granted from the Faculty Ethical Committee on Animal Experiments, University of Szeged.

Corticosterone and sesame oil were purchased from Sigma-Aldrich (St. Louis, MO, USA). The corticosterone was suspended in sesame oil at a volume adjusted to 1 ml/kg body weight (b.w.) and administered by subcutaneous injection (s.c.) into the nape of the neck. Control animals received the same amount of the vehicle (sesame oil). Experimental treatments were performed between 7.00 and 10.00 a.m. A total of 44 animals were used. To study the potential time-dependent, single-dose and chronic effects of corticosterone, experiments were performed in separate groups of animals. Depending on the experiment, treatments were given either as a single injection of 10 mg corticosterone, or chronically, as daily injections of 10 mg corticosterone for 21 consecutive days. The dose of 10 mg/day was selected consistently with previous studies showing changes in calcium homeostasis, including calcium-binding protein regulation in the hippocampus (Krugers et al., 1994, 1995).

In the part of experiment with single injection of corticosterone, rats were divided into two groups of five animals: rats treated with corticosterone and perfused 12 h after the injection and rats treated with corticosterone and perfused 24 h following treatment. The control groups (consisting of 5 animals each) received only the vehicle (sesame oil) and were perfused 12 and 24 h post-injection, respectively. In part of the study with chronically administered corticosterone, the

Table 1. Experimental groups, the number of animals and the immunohistochemical procedures performed in different groups.

Experiments	Corticosterone-treated animals (10 mg, s.c.)		Animals treated only with the vehicle (sesame oil; 1 ml/kg)	
Duration of treatment	Number of rats	Immunohistochemistry	Number of rats	Immunohistochemistry
12 h*	5	c-Fos, FosB	5	c-Fos, FosB
24 h*	5	c-Fos, FosB	5	c-Fos, FosB
21 days	12	FosB, CR, VIP, NPY	12	FosB, CR, VIP, NPY

*Post-injection time following the single injection of corticosterone and/or vehicle, when the animals were perfused.

experimental group (12 animals) received 10 mg/day corticosterone for 21 days, while the control group of 12 animals received only the vehicle (sesame oil). Twenty-four hours following the last injection the animals were perfused. Details of all groups are summarized in Table 1.

Prior to transcardial perfusion, rats were deeply anaesthetised with diethyl-ether and perfused with 0.1 M phosphate-buffered saline (PBS, pH 7.4), followed by 4% phosphate-buffered paraformaldehyde (pH 7.4) as a fixative. Brains were rapidly removed from the skull and post-fixed in 4% paraformaldehyde for 24 h at room temperature, before being cryoprotected overnight in 30% sucrose in PBS, pH 7.4. Serial coronal plane sections were cut at 24 μm thickness using a freezing microtome (Reichert-Jung, Cryocut 1800), and every fifth section was processed for immunohistochemistry.

Immunohistochemistry

c-Fos immunohistochemistry

Immunohistochemical labelling to localize c-Fos was performed as described below, using the streptavidin-peroxidase technique. Free-floating tissue sections were rinsed in PBS and subsequently pretreated for 10 min with 3% H_2O_2 and 0.5% Triton X-100 in PBS to block endogenous peroxidase. Sections were then rinsed in PBS followed by incubation for 1 h in 20% v/v normal pig serum (NPS, Sigma Chemical Co.) to suppress nonpecific antibody binding to the tissue. Then, brain sections were incubated overnight at room temperature with the primary polyclonal antisera against c-Fos (raised in rabbit; Santa Cruz Biotechnology, CA, USA) diluted 1: 2000 in 20% v/v NPS, and then in biotinylated donkey anti-rabbit IgG (Jackson Immuno-Research, USA), diluted 1:40 for 1 h. The secondary antibody was detected by incubating with streptavidin-peroxidase (Jackson Immuno-Research), diluted 1:1000 for 1 h. All dilutions and rinses between incubations were performed in PBS (pH 7.4). Finally, the enzyme label peroxidase was visualized by reaction with 0.05% diaminobenzidine tetrahydrochloride (DAB) containing 0.01% hydrogen peroxide and 0.3% nickel-sulphate (all reagents from Sigma) yielding a black reaction product. The sections were mounted on gelatine-coated slides, dehydrated in a graded ethanol series, cleared in xylene and coverslipped with Entellan (Fluka).

Immunodetection techniques to localize FosB, CR, VIP and NPY, respectively, were performed as described in detail here, the steps being identical and run simultaneously, with variations as described in the following sections.

FosB immunohistochemistry

Immunohistochemistry to detect FosB was performed as described in detail previously, except that the primary antibody against FosB was raised in rabbit (Santa Cruz Biotechnology, Santa Cruz, CA, USA), diluted 1:1000 in 20% v/v NPS, followed by biotinylated donkey anti-rabbit IgG (Jackson Immuno-Research, USA) diluted 1:40.

CR immunohistochemistry

Immunohistochemistry to detect CR was performed as described in detail previously, except that the primary antibody against CR was raised in goat (Santa Cruz Biotechnology, CA) diluted 1:3000 in 20% v/v NPS containing 0.03% thimerosal (Sigma). Sections were then incubated in biotinylated donkey-anti-goat IgG (diluted 1:400 in 10% v/v NPS).

VIP immunohistochemistry

Immunohistochemistry to detect VIP was performed as described in detail previously, except that the primary antibody against VIP was a polyclonal raised in rabbit (1:18000 dilution in 20% v/v NPS containing 0.03% thimerosal. The second layer was biotinylated anti-rabbit IgG raised in goat (1:400, in 10% v/v NPS).

NPY immunohistochemistry

Immunohistochemistry to detect NPY was performed as described in detail previously, except that the primary antibody against NPY was a polyclonal raised in rabbit (Santa Cruz Biotechnology, CA), diluted at 1:50.000 in 20% v/v NPS containing 0.03% thimerosal and the second layer was biotinylated donkey anti-rabbit IgG, diluted 1:400.

Negative controls

Controls were performed on free-floating sections. Labelling was performed exactly as described above, but primary antisera were omitted. No labelling was observed in these sections.

Analysis of the immunohistochemical data

Quantitative analysis was performed on five sections per animal from each experimental immunohistochemical group (see Table 1.), selected on the basis of identical stereotaxic coordinates (Paxinos and Watson, 1998). Areas of interest (AOIs) for immunoreactive cell counts were selected from the neocortex (CX) (S1Tr region), regions CA1 and CA3 of the hippocampus, and from the hilum and granule cell layer of the dentate gyrus (DG). The AOIs were determined using the rectangular image-capturing field of the camera. Within each AOI, the immunolabelled neuronal nuclei were counted using a Nikon Eclipse 600 microscope equipped with a SPOT RT Slider digital camera (1600 × 1200 dpi in 8 bits), using the Image ProPlus 4.0 morphometry software (Media Cybernetics, Silver Spring, MD, USA). Following background subtraction, the threshold was adjusted so that all labelled nuclei could be recognized. The counting was performed by an investigator blinded to the treatment group assignment.

In the CX, cell counts were performed using a 10 × objective magnification, and the AOI (an area of 0.2 mm^2) included each neocortical layer (I–VI), so that the layers were not evaluated separately. Then, cell counts were normalised to 1 mm^2. In the hippocampus, counting was performed using a 40 × objective. In areas CA1-3 of the Ammon's horn, the AOI (an area of 0.05 mm^2) included the *Stratum pyramidale*, and a narrow zone of the *Strata oriens* and *radiatum*. The hilum of the DG was outlined according to Amaral (1978), and used as AOI. The whole extent of the upper and lower blades of the granule cell layer was outlined and used as AOI, and immunolabelled nuclei were counted in this area. Cell counts were again normalised to 1 mm^2.

The data were analysed statistically comparing sets of findings obtained with the same magnification. ANOVA, followed by the Bonferroni post hoc test was used to determine differences in the number of immunoreactive nuclei in the control and corticosterone-treated animals. Significance level was $P < 0.05$.

Results

Effects of single-dose corticosterone administration on c-Fos immunoreactivity

c-Fos-immunoreactive cell nuclei were present in areas CA1-3 of the hippocampus, the granule cell layer of the DG, as well as in every layer of the CX 12 and 24 h following corticosterone administration. Most of the labelled nuclei in the Ammon's horn were detected in the pyramidal cell layer, which was strongly immunoreactive, whilst the *Strata oriens, radiatum* and *lacunosum-moleculare* contained a few, scattered c-Fos immunopositive nuclei. c-Fos immunopositive cell nuclei were observed in the granule cell layer of the DG, only weak c-Fos-immunopositivity was visible in the hilum. Accordingly, the cell nuclei in the hilum of the DG were not counted. Numerous, strongly labelled, activated cells were seen in layers II–VI of the CX 12 and 24 h following corticosterone treatment.

c-Fos-immunopositivity was not totally absent from these regions of the control animals receiving only the vehicle (sesame oil), since some scattered c-Fos-immunoreactive nuclei could be detected in the hippocampus, granule cell layer of the DG and in the CX.

Twelve hours following corticosterone administration, the pyramidal cell layer of the Ammon's horn and the dentate granule cell layer apparently contained more c-Fos immunopositive cell nuclei compared to control animals, and the number of immunoreactive nuclei was markedly higher in the region CA3, as confirmed by statistical evaluation. Moreover, corticosterone treatment resulted in significant increase in c-Fos immunopositive cell nuclei in the CX at 12 h after the injection, and the number of immunolabelled nuclei subsequently increased by 24 h. Quantification revealed that the number of c-Fos immunopositive cell nuclei significantly increased in CA1 and CA3 regions of the hippocampus, in the granule cell layer of the DG, as well as in the CX at 24 h post-injection, with respect to the control animals. Fos immunoreactivity in regions CA1 and CA3 of the Ammon's horn

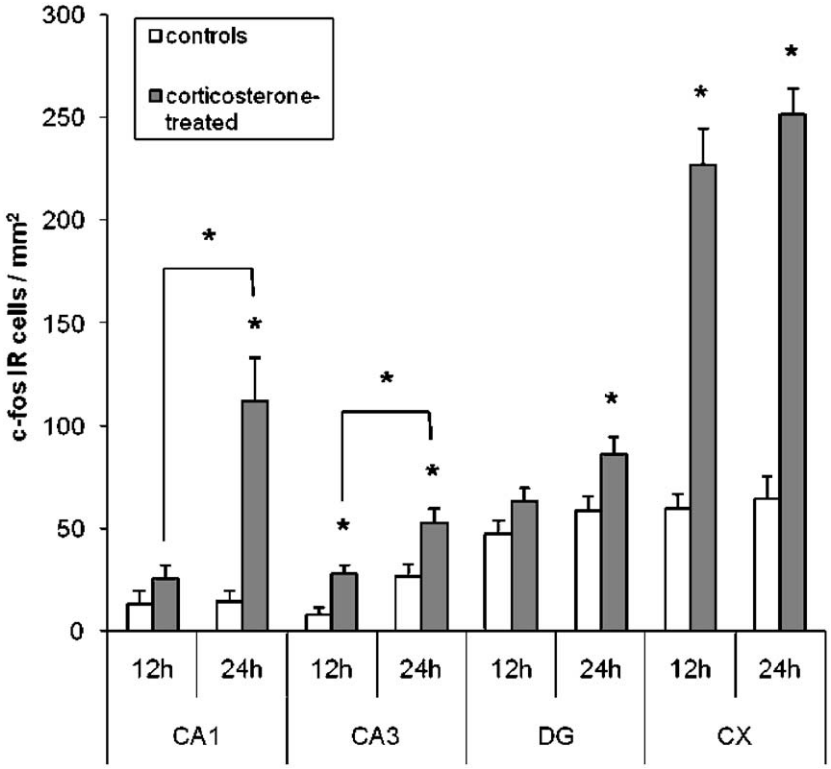

Figure 1. Results of c-Fos immunopositive cell counts in the hippocampus (CA1, CA3), in the dentate gyrus (DG) and the neocortex (CX) in corticosterone-treated rats 12 and 24 h post-injection compared with animals injected only with the vehicle (sesame oil). High dose of corticosterone significantly increased c-Fos immunopositivity in CA3 at 12 h, and it resulted in significant increase in c-Fos labelling in CA1, CA3 and DG by 24 h in comparison to controls. Note that hippocampal c-Fos immunoreactivity increased gradually between 12 and 24 h post-injection. Corticosterone treatment significantly increased neocortical c-Fos immunoreactivity 12 and 24 h post-injection. Asterisks denote significant differences ($p < 0.001$; ANOVA); the standard errors of the mean (SEM) are displayed as vertical bars.

increased gradually between 12 and 24 h post-injection (Figure 1).

Effects of single-dose and chronic corticosterone administration on FosB immunoreactivity

A low number of FosB immunolabelled cell nuclei were present in areas CA1-3 of the hippocampus, whereas the granule cell layer of the DG contained more numerous FosB immunoreactive nuclei in all investigated groups. The hilum was regularly devoid of FosB immunoreactivity by 12 and 24 h, similar to that observed in c-Fos immunolabelled samples. Repeated corticosterone treatment did not induce activation of the hilar cells. The CX displayed FosB immunolabelling in layers II–VI in acutely treated animals, with an overall decrease of labelled cells after chronic corticosterone administration.

Quantitative analysis revealed that application of corticosterone did not significantly alter FosB immonoreactivity in the hippocampus, the granule cell layer of the DG or the CX 12 or 24 h following single injection with respect to control animals. However, repeated administration of corticosterone for 21 days resulted in a significant increase of FosB immunolabelled cell nuclei in the granule cell layer of the DG. Three weeks of daily corticosterone treatment did not induce significant changes in the number of FosB immunoreactive cells in any of the hippocampal subfields.

Significant decrease of FosB immunolabelling was observed in the neocortical layers following chronic corticosterone administration (most pronounced decrease in layers II/III) compared to the number of immunoreactive cell nuclei at 12 h (Figure 2).

Effects of chronic corticosterone administration on CR, VIP and NPY immunoreactivity

The CR immunoreactive cell bodies were observed mainly in the pyramidal layer of the

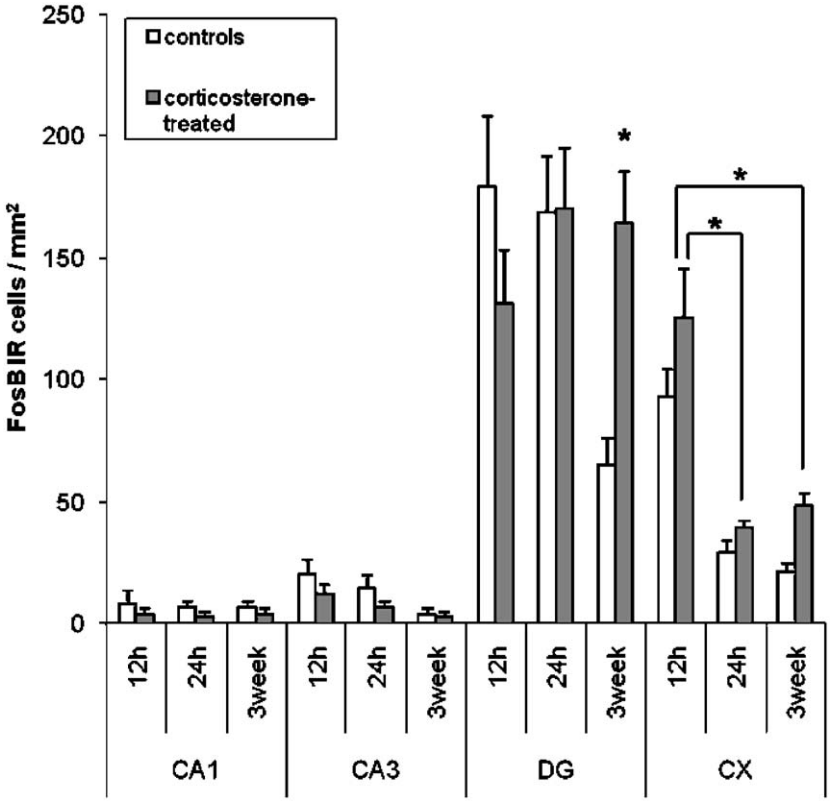

Figure 2. Effects of corticosterone treatment on FosB immunodetection in the hippocampus (CA1, CA3), in the dentate gyrus (DG) and the neocortex (CX). Rats were exposed to 12 and 24 h of high dose of corticosterone (single-dose), as well as to 3 weeks of daily corticosterone treatment. Control animals received only with the vehicle (sesame oil). Single-dose corticosterone administration did not cause any significant change. Chronic corticosterone treatment resulted in a significant increase in the number of FosB immunolabelled cell nuclei in DG. Asterisks: significant differences ($p < 0.001$; ANOVA); SEM is indicated in every case.

hippocampus, although some scattered cells were found in the inner zone of the *Stratum radiatum*, adjacent to the *Stratum lacunosum-moleculare*. CR immunopositive perikarya were most abundant in the *Strata pyramidale* of the CA1 and CA3 subfields of the Ammon's horn, and occasionally they could be found in the CA2 region, as well. Accordingly, we did not attempt to investigate the number of CR immunoreactive cells in the CA2 region separately; instead, cells were analyzed together in sectors CA1 and CA2 (CA1-2). The dendrites of the CR-positive neurons were located mainly in the *Stratum radiatum* and *lacunosum-moleculare*. In the DG, most of the CR-immunopositive cell bodies were found in the hilus; fewer, but still numerous CR-immunopositive neurons were seen in the granule cell layer. Most of the cells possessed long, thick, strongly labelled dendrites (Figure 3A). CR-immunopositive interneurons were also detected in layers II–VI of the CX.

Investigation of the number of CR-immunolabelled cells revealed that chronic corticosterone treatment did not change CR immunolabelling in sectors CA1-2 of the Ammon's horn, in the granule cell layer and the hilar region of the DG, and in the CX when compared to control animals (rats injected with sesame oil). However, 3 weeks of daily corticosterone treatment (10 mg/day, s.c.) significantly reduced the number of CR immunopositive cells in the CA3 area of the hippocampus (Figure 3B).

Following immunolocalisation of VIP, immunopositive neurons were seen in *Strata pyramidale* and *lacunosum-moleculare* of the Ammon's horn, most of the cell bodies being localized in region CA1, and less frequently in the CA3 area. VIP immunoreactive cell bodies were different in size and shape, as large multipolar, medium-sized and small oligopolar cells were also detectable. The majority of the VIP immunoreactive dendrites were long, rarely branching, with a straight course in the *Stratum radiatum*. VIP immunoreactive cells were present in the granule cell layer and in the hilum of the DG. A few, scattered cells were seen in the outer molecular layer (not counted). VIP immunopositive neurons were also visible in all layers of the CX.

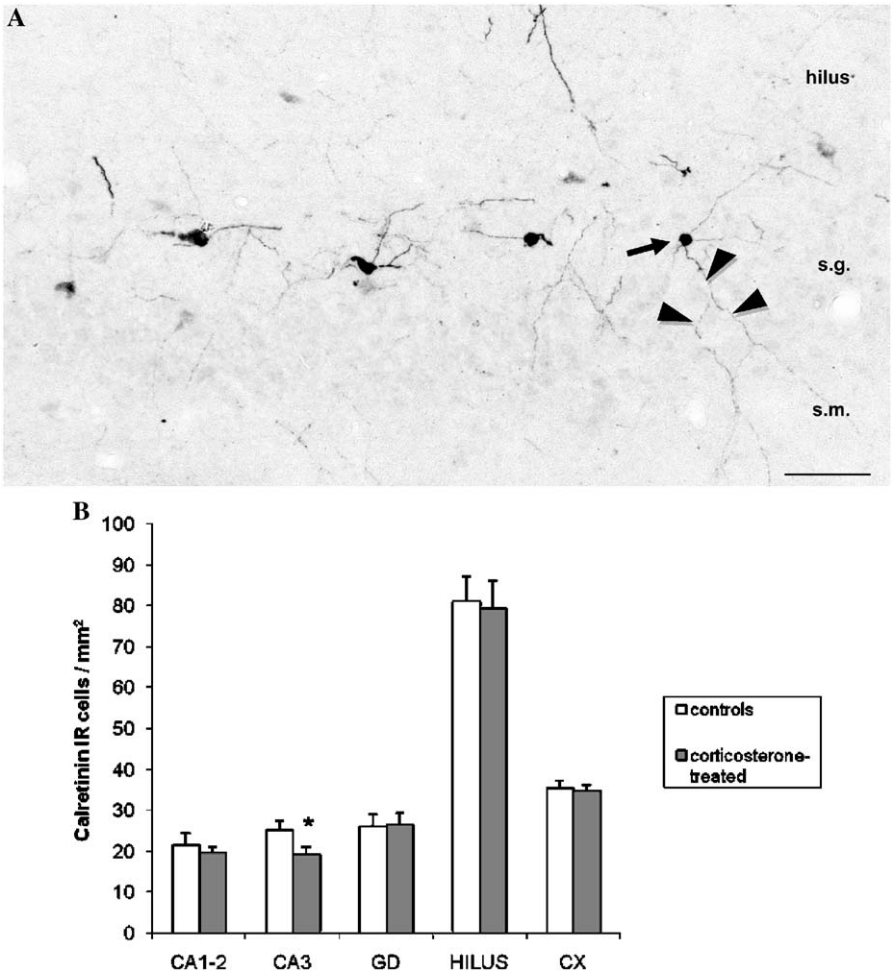

Figure 3. (A) Appearance of the CR immunolabelling in the DG. The arrow indicates a CR immunoreactive cell body, while arrowheads point to the dendrites of the cell; s.g.: *Stratum granulosum*, s.m.: *Stratum moleculare*. Bar: 100 μm. (B) Results of CR-immunopositive cell counts in the Ammon's horn (CA), in the granule layer of the dentate gyrus (GD) and in the hilum (HILUS), as well as in the neocortex (CX) in corticosterone-treated animals compared with rats injected with the vehicle. Three weeks of corticosterone treatment decreased the number of CR immunopositive neurons exclusively in the CA3 region. Significant differences are shown by asterisks ($P < 0.005$). The standard error of the mean is displayed at the top of the columns.

Three weeks of treatment with a high dosage of corticosterone (10 mg/day) did not affect the number of VIP immunoreactive neurons in the above regions (Figure 4). No changes in VIP immunolabelling pattern were found in the hippocampus, in the DG or in the CX after 3 weeks of daily corticosterone treatment.

NPY-immunoreactive neurons were located in *Strata pyramidale* and *oriens* of the CA1-3 subfields of the Ammons's horn; smaller number of cells was also present in *Stratum radiatum*, with higher frequency close to the granule cell layer. *Stratum lacunosum-moleculare* was regularly devoid of NPY immunopositive cell bodies. Most of the NPY-containing perikarya were partially labelled, with different morphological features (large multipolar, pyramidal shaped and fusiform cell bodies were also distinguishable). In the DG, the majority of the NPY-immunoreactive neurons were seen in the hilum and at the base of the granule cell layer (bordering the hilum). NPY immunopositive neurons were present in all layers of the CX. Quantification revealed that chronic corticosterone treatment did not alter NPY immunoreactivity in the hippocampus and the DG. However, a significant reduction of NPY immunopositive interneurons in the CX was found after three weeks of daily costicosterone treatment (Figure 5).

Discussion

Our previous results were in accord with data in the literature concerning the detection and

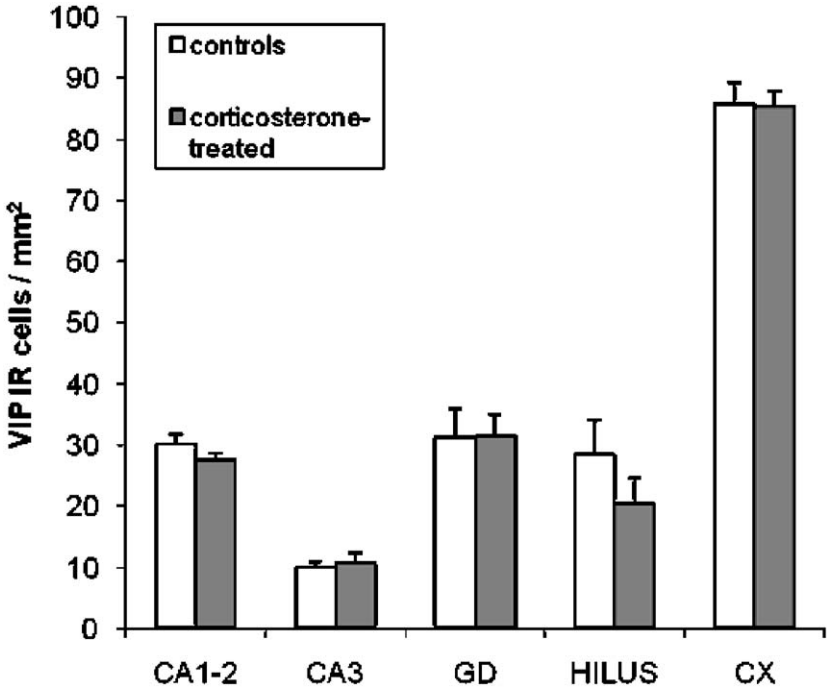

Figure 4. VIP immunoreactive neurons in the Ammon's horn (CA), in the granular layer of the dentate gyrus (GD), in the hilum (HILUS) and in the neocortex (CX) in rats treated for three weeks with corticosterone (10 mg/day, s.c.) and in control animals (given only the vehicle). Three weeks of treatment with corticosterone did not significantly alter the number of VIP-immunoreactive neurons.

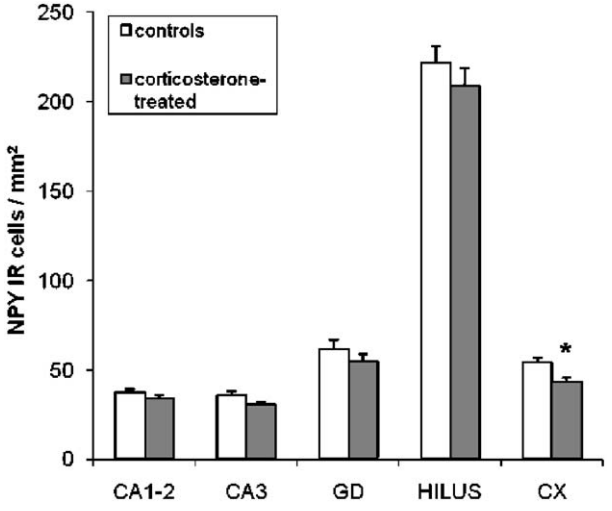

Figure 5. NPY-immunoreactive neurons in the Ammon's horn (CA), in the granular layer of the dentate gyrus (GD), in the hilum (HILUS) and in the neocortex (CX) in corticosterone-treated rats in comparison with control animals. Three weeks of treatment with corticosterone significantly decreased NPY immunoreactive cell number in the CX ($P<0.005$).

evaluation of Fos immunoreactivity as a marker of neuronal activation (Mihály et al., 2001; Szakács et al., 2003; Fazekas et al., 2006). Expression of c-*Fos* is induced mainly by transmitter-mediated input and voltage-dependent Ca^{2+} currents (Greenberg and Ziff, 2001), and thereby Fos protein detection is a recognized marker of rapid changes in neuronal activity, reliable and widely used for pharmacological investigations (Campeau et al., 1997; Skorzewska et al., 2006). A brief activation of glucocorticoid receptors occurring at high corticosterone levels leads to enhanced Ca^{2+} influx into hippocampal cells, mediated principally by voltage-gated Ca^{2+} channels (Joels, 2001). There is evidence that acute administration of high doses of corticosterone or exposure to various acute stressors affects (among emotional, behavioural and hormonal responses) Ca^{2+}-mediated neuronal responses, including immediate-early gene, such as c-*Fos* gene, and thereby Fos protein localisation, within several brain regions with a focus on subcortical limbic structures (Melia et al., 1994; Cullinan et al., 1995; Chowdhury et al., 2000; Skorzewska et al., 2007). In the present study, we found that acutely administered high-doses of corticosterone (10 mg s.c.) increased c-Fos immunolabelling in the hippocampus in a time-dependent manner, as hippocampal c-Fos immunoreactivity increased gradually between 12 and 24 h after corticosterone injection, and was highest at 24 post-injection. This overall increase in the number of c-Fos-immunopositive cells in the CA1 and CA3 subfields of the hippocampus is consistent with other studies finding a significant induction of

c-*Fos* gene and Fos protein detection in the hippocampus in response to acute stressors, although the pattern and the time course of immediate early gene and protein detection varies with stimulus type and intensity (Cullinan et al., 1995; Campeau and Watson, 1997; Chowdhury et al., 2000; Pace et al., 2005). Acute stress-induced c-*Fos* mRNA has been reported to reach peak levels at 60 min and is markedly reduced at 2 h post-stress; however, in some cases, a delayed and/or prolonged induction was detected, which may be indicative of secondary neuronal activation in neocortical and allocortical regions (Cullinan et al., 1995). Our present experiments might provide evidence that acute administration of a high dose of corticosterone (10 mg. s.c) can lead to delayed and/or prolonged *Fos* expression in the hippocampal formation (12 and 24 h, respectively), probably through secondary neuronal activation. However, this issue should be proved by further experiments by means of in-situ hybridization or Northern blotting of specific mRNA. It is noteworthy that we used different experimental paradigm and importantly higher dose of corticosterone, as the dose of 5 mg/kg was found in previous studies to increase plasma corticosterone levels in a manner similar to the effect of an acute stressor (Skorzewska et al., 2006). Interestingly, some studies assessing acute stress-associated *Fos* expression in the hippocampal subregions found rapid c-*Fos* induction and increased levels of Fos protein in the CA1-CA2 and CA3 areas, as well as in the inner blade of the DG, whereas Fos decreased in the outer blade of the DG (Pace et al., 2005). This special and unusual pattern of changes, a decrease in DG Fos following acute (restraint) stress, has been reported also in other studies (Chowdhury et al., 2000; Fevurly and Spencer, 2004). Although we did not count the number of activated cells separately in the DG subfields, we saw a somewhat similar pattern of dentate Fos immunodetection 12 h after corticosterone injection. Whereas high levels of corticosterone resulted in visibly enhanced c-Fos immunodetection in the inner blade of the GD, it resulted in less c-Fos immunopositive cells in the outer blade. However, the total number of activated cells was slightly, but not significantly, higher 12 h post-injection, and subsequently increased at 24 h.

Our results indicate that activation of region CA3 occurs at 12 h after corticosterone injection, followed by activation of the DG and region CA1, as well as further activation of region CA3 by 24 h. The CA3 area therefore preceded DG as relates to the appearance of c-Fos protein. One explanation of this observation may be a delayed and probably divergent and less effective activation of the entorhinal afferents, compared to the pattern and time course of cell activation at 1–3 h following acute stress or other factors enhancing presynaptic glutamate release (Cullinan et al., 1995; Mihály et al., 2001). There is extensive evidence that glucocorticoids and stress itself causes glutamate release in the hippocampus (for a review see: Sapolsky et al., 1995). When activated, the glutamatergic perforant pathway projects to the DG and also to the pyramidal neurons of regions CA3 and CA1 (Jones, 1993), and also impinges on inhibitory interneurons of the Ammon's horn (Freund and Buzsáki, 1996; Gulyás et al., 1999). Moreover, the mossy fibers innervate not only the CA3 principal cells, but also the inhibitory interneurons of the hilus and region CA3 (Acsády et al., 1998), thus exerting a feed-forward inhibition on the CA3 pyramidal cells. This complex circuitry might explain our observation regarding the differences in c-Fos immunolocalisation in region CA3 with respect to the DG and region CA1 at 12 h post-injection. At very high corticosteroid levels, there is supposed to be a direct suppression of excitatory glutamate-mediated input, suppressing temporarily the transfer of excitatory input through the hippocampus (Joels, 2001). Such suppression of excitatory glutamatergic neurotransmission through entorhinal afferents might consequently diminish or abolish mossy fiber-driven inhibitory control over CA3 principal cells, leading to activation of c-*Fos*. Further exposure to high corticosterone level might disrupt the temporary suppression of excitatory input, as well as this hypothesized disinhibition mechanism, resulting in the activation of the DG and, thereby, in the activation of regions CA3 and CA1 at 24 h.

Our findings are in accordance with data in the literature as concerns the appearance of *Fos* expression in the CX related to acutely increased corticosterone levels, such as seen following acute stress or after acute corticosterone administration. It is notable that most of these data refer to the alterations of neuronal activity and associated immediate early gene c-*Fos* expression in a well-defined, short time-course. It was shown, for example, that exposure to stressful novel experiences led to an increase in c-*Fos* expression in primary motor as well as primary somatosensory cortex after 15–60 min of exposure to stress (Pace et al., 2005). Similarly, a dramatic induction of c-*Fos* mRNA was observed in several neocortical regions in response to acute stress, with peak levels at 0.5–1 h, markedly reduced at 2 h post-stress (Cullinan et al., 1995). We found that single injection of high dose of corticosterone induced a

substantial increase in c-Fos protein localisation in the CX (area selected from the somatosensory region) 12 and 24 h post-injection. This result is consistent with other studies as concerns c-Fos in cortical areas 1 day following acute corticosterone administration; however, enhanced activity was found in the cingulated cortex (Skorzewska et al., 2007). In another experiment, acute, mild and severe stress-induced increase in Fos was detected in the somatosensory cortex, whereas Fos levels in the cingulated cortex depended on the severity of acute stress (Chowdhury et al., 2000). These data indicate that neocortical and allocortical regions are sensitive to adaptation to acute stress and/or enhanced corticosterone levels. The present results might provide further evidence to a delayed neuronal activation of the CX in response to acutely increased corticosterone levels.

When reflecting on the overall low level of FosB labelling in the hippocampus and the somatosensory cortex 12 and 24 h following a single injection of high dose of corticosterone, we considered the possibility that this might be a result of an important distinction between corticosterone-mediated inductions of the members of the *Fos* gene families, such as c-*Fos* and *FosB*. The discrepancy between the region and time-dependent transcriptional profiles of c-*Fos* and *FosB* after acute corticosterone challenge has recently been reported, as a widespread and transient reduction of *FosB* mRNA was found in the hippocampus 0.8 h after acute corticosterone injection, whereas changes in c-*Fos* levels were restricted to the DG (Hansson and Fuxe, 2008). In the same experiment, after 24 h of corticosterone exposure a reduction of *FosB* mRNA was again detected in CA1 with trends toward reduction in other hippocampal regions and the somatosensory cortex. In our experiments, FosB levels seem to be unaffected by acute corticosterone treatment 12 and 24 h post-injection, not only in the CA1 and CA3 hippocampal subfields and in the DG, but also in the somatosensory cortex. However, a slight, but not significant decrease of FosB labelling could be observed in the CA1 and CA3 regions and a time-dependent significant reduction of FosB immunoreactivity in the CX; this observation is in accordance with the recent results of Hansson and Fuxe (2008). Overall, discrepancies among hippocampal and neocortical regions were observed in the present study in terms of acute corticosterone-induced changes in *Fos* family distributon. While acute corticosterone injection increased the number of c-Fos-immunoreactive cell nuclei in the hippocampus and the neocortex at 24 h, it did not significantly affect FosB immunoreactivity in the hippocampal subregions and caused a time-dependent reduction of FosB immunpositivity in the somatosensory cortex. These observations demonstrate that a single, high dose of corticosterone can cause a differential acute alteration of c-Fos and FosB levels in the hippocampus and the neocortex, which may reveal special acute plasticity changes in these structures.

When investigating the impact of repeated administration of a high dose of corticosterone on immediate early gene *FosB* expression, we found that prolonged treatment with steroid hormone enhanced FosB immunoreactivity in the DG, while FosB immunolocalisation was unaffected in CA1 and CA3 hippocampal subfields and in the somatosensory cortex. It is noteworthy that neocortical FosB immunodetection was significantly reduced in chronically corticosterone-treated rats compared to a single acute administration (12 h post-injecton). Previous studies have shown that repeated stressful stimuli increase the expression of the transcription factor FosB in limbic structures and associated regions, and interestingly, constitutive absence of FosB influences stress-related behavioral traits, as *FosB* knockout mice exhibit pronounced behavioral abnormalities when stress levels are high (Zhu et al., 2007). Both chronic stress and chronic administration of corticosterone produce deficits in cognition and behavior, accompanied by synaptic plasticity changes in corticolimbic structures, including alterations in neural function, chemistry and morphology (Woolley et al., 1990; Watanabe et al., 1992; Luine et al., 1993; Lyons et al., 2000; Wellmann, 2001). Accordingly, chronic administration of a high dose of corticosterone (s.c. injections of 10 mg corticosterone, daily for 3 weeks, similar experimental paradigm as used in our present experiments) was demonstrated to reorganize dendritic arborisation in pyramidal neurons in medial prefrontal cortex, reflecting functional changes in corticolimbic structures (Wellmann, 2001). Chronic corticosterone treatment was found to produce a complex pattern of changes in c-*Fos* expression, as repeatedly administered steroid hormone increased c-Fos immunolabelling in the CA1 and CA2 areas of the hippocampus, unaffected in the CA3 region, and decreased the aversive context-induced c-Fos production in the DG (Skorzewska et al., 2006). However, the impact of a chronic, high dose of corticosterone treatment on immediate early FosB immunopositivity is first reported in the present study. Chronic corticosterone treatment (10 mg s.c.) significantly enhanced FosB labelling in the DG, but not in the CA1 and CA3 subfields of the hippocampus. This finding might be of special interest given the assumed role of the hippocampus, and

particularly the DG in adaptive mechanisms and prolonged plasticity changes. Considering the role of inhibitory neurons in the control of synaptic mechanisms in the hippocampus, we assessed the effects of a prolonged, high dose of corticosterone on specific populations of interneurons. Three weeks of corticosterone treatment decreased the number of CR immunopositive neurons exclusively in the CA3 region, did not affect VIP immunoreactivity and reduced the number of NPY-immunopositive neurons in the CX. Chronic treatment with a high dosage of corticosterone did not change CR immunoreactivity in the granular layer and the hilus of the DG when compared to control animals. These observations concerning the DG closely resemble previous investigations on the calcium-binding protein, calbindin, immunoreactivity following chronic corticosterone treatment, using the same dose of steroid hormone (three weeks daily 10 mg s.c.; Krugers et al., 1994, 1995). It is noteworthy, that in these experiments, 3 weeks of corticosterone treatment was found to increase calbindin immunoreactivity in the CA1 pyramidal layer, with no degenerative changes in CA3 area (Krugers et al., 1995). In the present experiment, chronic corticosterone treatment significantly and selectively attenuated CR immunoreactivity in the CA3 area. The finding that a high dosage of corticosterone affected CR immunoreactivity in the CA3 subfield is in accordance with a previous observation, by Krugers et al. (1995), that CA3 interneurons degenerate 3 weeks following corticosterone treatment (Pavlides et al., 1993). Accordingly, it has been reported that exposure to 3 weeks of corticosterone treatment or stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons and affects hippocampal physiology and LTP in the CA3 area (Woolley et al., 1990; Pavlides et al., 2002). It was also found that high dose of corticosterone (10 mg/day) reduced CR mRNA levels in several discrete brain areas (Strauss et al., 1995), indicating that the impact of corticosterone on brain CR is related to its pathological effects, including neuronal energy depletion or interference with normal glial functions. This might explain our present findings that high dosage of chronic corticosterone treatment decreased CR immunoreactivity in the CA3 area, suggesting the pathological effect of steroid hormone on CR in this vulnerable hippocampal subfield. It remains, however, to be determined why this effect was exclusively seen in the CA3 area and not in other hippocampal subfields or in the DG.

Despite the co-localization of VIP and the calcium-binding protein, CR, in a subpopulation of VIP immunoreactive interneurons in the hippocampus (Acsády et al., 1996), we did not find any change in the number of VIP immunopositive cells in response to chronic corticosterone treatment. Interestingly, about 50% of the VIP immunoreactive cells in CA1 and CA3 were shown to be immunopositive for CR. It should be noted at the same time, that interneurons co-localizing CR and VIP represent about 30% of CR containing cells in the CA1 area, but only approximately 10% in the CA3 subfield (Acsády et al., 1996). This relatively small proportion of the CR containing neurons in the CA3 area might partially explain the discrepancy between the effects of corticosterone on CR and VIP immunoreactivity. However, this issue should be further elucidated using a double labelling technique. The other reason may be the different impact of corticosterone on CR- and neuropeptide-containing interneurons in this hippocampal subfield. This assumption is supported by the observation that the number of VIP and NPY-immunoreactive interneurons was unaffected in CA3 area following administration of high doses of corticosterone. Nevertheless, chronic corticosterone treatment did not alter the number of CR-, VIP- and NPY-containing interneurons in the granule layer or hilum of the DG.

In summary, the present immunohistochemical data indicate that apart from the acute effects of the steroid hormone, chronic corticosterone treatment (10 mg/day) (i) enhanced FosB immunoreactivity in the DG and did not affect the number of CR-, VIP- and NPY-immunopositive interneurons in the dentate granule layer and the hilus, and (ii) high dose of corticosterone appears to affect CR immunoreactivity in the CA1 area. These observations might help to supplement literature data over mechanisms related to special plasticity changes in the hippocampus induced by exposure to high doses of corticosterone.

Acknowledgements

This study was supported by the Hungarian National Research Fund and by the Ministry of Education (OTKA T 046152/2004).

References

Acsády L, Arabadzisz D, Freund TF. Correlated morphological and neurochemical features identify different subsets of vasoactive intestinal polypeptide-immunoreactive interneurons in rat hippocampus. Neuroscience 1996;73:299–315.

Acsády L, Kamondi A, Sík A, Freund T, Buzsáki G. GABAergic cells are the major postsynaptic targets of mossy fibers in the rat hippocampus. J Neurosci 1998;18:3386–403.

Amaral DG. A Golgi study of cell types in the hilar region of the hippocampus in the rat. J Comp Neurol 1978; 182:851–914.

Ayroldi E, Zollo O, Macchiarulo A, Di Marco B, Marchetti C, Riccardi C. Glucocorticoid-induced leucine zipper inhibits the Raf-extracellular signal-regulated kinase pathway by binding to Raf-1. Mol Cell Biol 2002;22: 7929–41.

Campeau S, Watson SJ. Neuroendocrine and behavioural responses and brain pattern of c-Fos induction associated with audiogenic stress. J Neuroendocrinol 1997;9:577–88.

Campeau S, Falls WA, Cullinan WE, Helmreich DL, Davis M, Watson SJ. Elicitation and reduction of fear: Behavioural and neuroendocrine indices and brain induction of the immediate-early gene c-Fos. Neuroscience 1997;78:1087–104.

Chowdhury GM, Fujioka T, Nakamura S. Induction and adaptation of Fos expression in the rat brain by two types of acute restraint stress. Brain Res Bull 2000;52: 171–82.

Cullinan WE, Herman JP, Bataglia DF, Akil H, Watson SJ. Pattern and time course of immediate early gene expression in rat brain following acute stress. Neuroscience 1995;64:477–505.

Czyrak A, Mackowiak M, Chocyk A, Fijal K, Tokarski K, Bijak M, Wedzony K. Prolonged corticosterone treatment alters the responsiveness of 5-HT1A receptors to 8-OH-DPAT in rat CA1 hippocampal neurons. Naunyn Schmiedebergs Arch Pharmacol 2002;366:357–67.

De Kloet ER. Brain corticosteroid receptor balance and homeostatic control. Front Neuroendocrinol 1991;12: 95–164.

Fazekas I, Szakács R, Mihály A, Zádor Zs, Krisztin-Péva B, Juhász A, Janka Z. Alterations of seizure-induced c-Fos immunolabelling and gene expression in the rat cerebral cortex following dexamethasone treatment. Acta Histochem 2006;108:463–73.

Fevurly RD, Spencer RL. Fos expression is selectively and differentially regulated by endogenous glucocorticoids in the paraventricular nucleus of the hypothalamus and the dentate gyrus. J Neuroendocrinol 2004;16: 970–9.

Freund TF, Buzsáki G. Interneurons of the hippocampus. Hippocampus 1996;6:347–470.

Gould E, Tanapat P. Stress and hippocampal neurogensis. Biol Psychiatry 1999;46:1472–9.

Greenberg ME, Ziff EB. Signal transduction in the postsynaptic neuron. Activity-dependent regulation of gene expression. In: Cowan WM, Südhof TC, Stevens CF, editors. Synapses. Baltimore: The John Hopkins University Press; 2001. p. 357–91.

Gulyás AI, Megías M, Emri Zs, Freund TF. Total number and ratio of excitatory and inhibitory synapses converging onto single interneurons of different types in the CA1 area of the hippocampus. J Neurosci 1999; 19:10082–97.

Haller J, Halasz J, Makara GB, Kruk MR. Acute effects of glucocorticoids: Behavioral and pharmacological perspectives. Neurosci Biobehav Rev 1998;23:337–44.

Hansson AC, Fuxe K. Time-course of immediate early gene expression in hippocampal subregions of adrenalectomized rats after acute corticosterone challenge. Brain Res 2008;1215:1–10.

Herdegen T, Leah JD. Inducible and constitutive transcription factors in the mammalian nervous system: Control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. Brain Res Rev 1998;28:370–490.

Joëls M. Corticosteroid actions in the hippocampus. J Neuroendocrinol 2001;13:657–69.

Jones RSG. Entorhinal-hippocampal connections: A speculative view of their function. Trends Neurosci 1993; 16:58–64.

Karst H, Wadman WJ, Joëls M. Corticosteroid receptor-dependent modulation of calcium currents in rat hippocampal CA1 neurons. Brain Res 1994;649:234–42.

Kawato S, Yamada M, Kimoto T. Neurosteroids are 4th generation neuromessengers: Cell biophysical analysis of steroid transduction. Adv Biophys 2001;37:1–30.

Kerr DS, Campbell LW, Thibault O, Landfield PW. Hippocampal glucocorticoid receptor activation enhances voltage-dependent Ca conductances: relevance to brain aging. Proc Natl Acad Sci USA 1992;89: 8527–31.

Krugers HJ, Medema RM, Postema F, Korf J. Calbinidin-D28 K and parvalbumin immunoreactivity in the rat hippocampus following adrenalectomy and corticosterone treatment. Ann N Y Acad Sci 1994;746:463–6.

Krugers HJ, Medema RM, Postema F, Korf J. Region-specific alterations of calbindin-D28k immunoreactivity in the rat hippocampus following adrenalectomy and corticosterone treatment. Brain Res 1995;696: 89–96.

Luine VN, Spencer RL, McEwen BS. Effect of corticosterone ingestion on spatial memory performance and hippocampal serotonergic function. Brain Res 1993; 616:55–70.

Lyons DM, Lopez JM, Yang C, Schatzberg AF. Stress-level cortisol treatment impairs inhibitory control of behaviour in monkeys. J Neurosci 2000;20:7816–21.

McEwen BS, de Kloet ER, Rostene W. Adrenal steroid receptors and actions in the nervous system. Physiol Rev 1986;66:1121–88.

Melia K, Ryabinin A, Schroeder R, Bloom F, Wilson M. Induction and habituation of immediate early gene expression in rat brain by acute and repeated restraint stress. J Neurosci 1994;14:5929–38.

Mihály A, Szakács R, Bohata Cs, Dobó E, Krisztin-Péva B. Time-dependent distribution and neuronal localization of c-Fos protein in the rat hippocampus following 4-aminopyridine seizures. Epilepsy Res 2001;44: 97–108.

Morgan JI, Curran TE. Proto-oncogenes. Beyond second messengers. Psychopharmacology: The Fourth Generation

of Progress. In: Bloom FE, Kupfer DJ, editors., New York: Raven Press; 1995.

Pace TWW, Gaylord R, Topczewski F, Girotti M, Rubin B, Spencer RL. Immediate-early gene induction in hippocampus and cortex as a result of novel experience is not directly related to the stressfulness of that experience. Eur J Neurosci 2005;22:1679–90.

Pavlides C, Magarinos A, Gould E, Hsu M, Pierre E, Watanabe Y, McEwen BS, Buzaki G. Adrenalectomy induced degeneration of granule cells but not CA3 interneurons is prevented by corticosterone treatment. Soc Neurosci Abstr 1993;19:168.

Pavlides C, Nivón LG, McEwen BS. Effects of chronic stress on hippocampal long-term potentiation. Hippocampus 2002;12:245–57.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. San Diego: Academic Press; 1998.

Rajkowska G. Postmortem studies in mood disorders indicate altered numbers of neurons and glial cells. Biol Psychiatry 2000;48:766–77.

Reul JM, Van den Bosch FR, De Kloet ER. Relative oocupation of type-I and type-II corticosteroid receptors in rat brain following stress and dexamethasone treatment: Functional implications. J Endocrinol 1987; 115:459–67.

Sapolsky RM. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry 2000; 57:925–35.

Sapolsky R, Brooke S, Stein-Behrens B. Methodologic issues in studying glucocorticoid-induced damage to neurons. J Neurosci Methods 1995;58:1–15.

Sato S, Osanai H, Monma T, Harada T, Hirano A, Saito M, Kawato S. Acute effect of corticosterone on *N*-methyl-D-aspartate receptor-mediated Ca^{2+} elevation in mouse hippocampal slices. Biochem Biohys Res Com 2004;321:510–3.

Shibuya K, Takata N, Hojo Y, Furukawa A, Yasumasu N, Kimoto T, Enami T, Suzuki K, Tanabe N, Ishii H, Mukai H, Takahashi T, Hattori T, Kawato S. Hippocampal cytochrome P450s synthesize brain neurosteroids which are paracrine neuromodulators of synaptic signal transduction. Biochem Biophys Acta 2003;1619: 301–16.

Skorzewska A, Bidzinski A, Lehner M, Turzynska D, Wislowska-Stanek A, Sobolewska A, Szyndler J, Maciejak P, Taracha E, Plaznik A. The effects of acute and chronic administration of corticosterone on rat behaviour in two models of fear responses, plasma corticosterone concentration, and c-Fos expression in the brain structures. Pharmacol, Biochem Behav 2006;85:522–34.

Skorzewska A, Bidzinski A, Lehner M, Turzynska D, Sobolewska A, Hamed A, Szyndler J, Maciejak P, Plaznik A. The effects of acute corticosterone administration on anxiety, endogenous corticosterone, and c-Fos expression in the rat brain. Horm Behav 2007; 52:317–25.

Strauss KI, Schulkin J, Jacobowitz DM. Corticosterone effects on rat calretinin mRNA in discrete brain nuclei and the testes. Brain Res Mol Brain Res 1995;28:81–6.

Szakács R, Weiczner R, Mihály A, Krisztin-Péva B, Zádor Zs, Zádor E. Non-competitive NMDA receptor antagonists moderate seizure-induced c-*Fos* expression in the rat cerebral cortex. Brain Res Bull 2003;59:485–93.

Vidal C, Jordan W, Zieglgansberger W. Corticosterone reduces the excitability of hippocampal pyramidal cells *in vitro*. Brain Res 1986;383:54–9.

Watanabe Y, Gould E, McEwen BS. Stress induces atrophy of apical dendrites of hippocampal pyramidal neurons. Brain Res 1992;588:341–5.

Wellmann CL. Dendritic reorganization in pyramidal neurons in medial prefrontal cortex after chronic corticosterone administration. J Neurobiol 2001;49: 245–53.

Wiegert O, Pu Z, Shor S, Joëls M, Krugers H. Glucocorticoid receptor activation selectively hampers *N*-methyl-D-aspartate receptor dependent hippocampal synaptic plasticity *in vitro*. Neuroscience 2005;135: 403–11.

Wolkowitz OM, Epel ES, Reus VI. Stress hormone-related psychopathology: Pathophysiological and treatment implications. Biol Psychiatry 2001;2:115–43.

Woolley CS, Gould E, McEwen BS. Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. Brain Res 1990;531: 225–31.

Zhu H, Lee M, Agatsuma S, Hiroi N. Pleiotropic impact of constitutive FosB inactivation on nicotine-induced behavioral alterations and stress-related traits in mice. Hum Mol Genet 2007;16:820–36.