c-fos Reduces Corticosterone-Mediated Effects on Neurotrophic Factor Expression in the Rat Hippocampal CA1 Region

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The transcription of neurotrophic factors, i.e., basic fibroblast growth factor (*bFGF*) and brain-derived neurotrophic factor (*BDNF*) is regulated by glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) activation despite the lack of a classical glucocorticoid response element in their promoter region.

A time course for corticosterone (10 mg/kg, s.c.) in adrenalectomized rats revealed a peak hormone effect at the 4 hr time interval for *bFGF* (110–204% increase), *BDNF* (53–67% decrease), *GR* (53–64% decrease), and *MR* (34–56% decrease) mRNA levels in all hippocampal subregions using *in situ* hybridization. *c-fos* mRNA levels were affected exclusively in the dentate gyrus after 50 min to 2 hr (38–46% decrease).

Furthermore, it was evaluated whether corticosterone regulation of these genes depends on interactions with the transcription factor complex activator protein-1. *c-fos* antisense oligodeoxynucleotides were injected into the dorsal hippocampus of adrenalectomized rats. Corticosterone was given 2 hr later, and the effects on gene expression were measured 4 hr later. In CA1, antisense treatment significantly and selectively enhanced the hormone action on the expression of *bFGF* (44% enhanced increase) and *BDNF* (38% enhanced decrease) versus control oligodeoxynucleotide treatment. In addition, an upregulation of *c-fos* expression (89% increase) was found. There were no effects of *c-fos* antisense on hippocampal *GR* and *MR* expression. Thus it seems that a tonic *c-fos* mechanism exists within CA1, which reduces *GR*- and *MR*-mediated effects on expression of *bFGF* and *BDNF*.

Key words: adrenalectomy; basic fibroblast growth factor; brain-derived growth factor; glucocorticoid receptor; mineralocorticoid receptor; immediate early gene; antisense oligodeoxynucleotides; *in situ* hybridization; rat brain

Introduction

The adrenal steroid hormone corticosterone (CORT) regulates transcription via activation of two types of corticosteroid receptors, namely the mineralocorticoid receptor [MR (or type I)] and the glucocorticoid receptor [GR (or type II)], which can act directly as transcription factors by binding to their common glucocorticoid response element (GRE) (Beato et al., 1995). However, although there are clearly documented CORT effects on transcription of a number of genes, among them *GR* and *MR* as well as basic fibroblast growth factor (*bFGF*) and brain-derived neurotrophic factor (*BDNF*) (Barbany and Persson, 1992; Chao et al., 1998; Hansson et al., 2000), no classical GREs have been found within the promoter regions of these genes. Thus, *GR* and *MR* may also interact with other regulators of transcription either by competition for shared binding motifs (Diamond et al., 1990; Funder, 1993; Pearce and Yamamoto, 1993) or by protein–protein interactions with other transcription factors (Schüle et al.,

1990; Yang-Yen et al., 1990; König et al., 1992; Reichardt et al., 1998).

The *GR* and the transcription factor activator protein-1 (*AP-1*) complex appear to interact with each other via mechanisms both dependent on and independent from DNA binding (Diamond et al., 1990; Schüle et al., 1990; Yang-Yen et al., 1990; König et al., 1992). *AP-1* complexes consisting of *c-fos*/*c-jun* or *c-fos*/*junB* heterodimers are transcriptionally highly active. Tissue culture experiments have shown a negative interaction of *GR* and *AP-1* complexes on the transcriptional activity of target genes (Diamond et al., 1990; Jonat et al., 1990; Lucibello et al., 1990), but such interactions have not been observed for *MR* (Pearce and Yamamoto, 1993; Pearce, 1994). However, the importance of *c-fos* in the adrenal steroid hormone regulation in the brain has never been studied *in vivo*.

With use of the adrenalectomized (ADX) animal model, it has been shown recently by means of *in situ* hybridization that CORT affects neurotrophic factor gene mRNA levels in the dorsal hippocampus that are unique for each particular gene in a cellular and region-specific manner and therefore seem to be strongly context dependent (Hansson et al., 2000). Here we ask whether the pattern of CORT-induced gene expression of *bFGF* and *BDNF* in the hippocampal subregions may be the result, in part, of *GR*/*MR* or *AP-1* interactions, or both. Toward this aim, the

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ADX rat was used; this allows an analysis of the effects of an exogenous hormonal stimulus without interference with the endocrine stress response of the animals. The time course of gene expression was analyzed in the dorsal hippocampus for the neurotrophic factors *bFGF* and *BDNF*, for the corticosteroid receptors *GR* and *MR*, and for *c-fos*, a highly active component of the AP-1 complex, in response to CORT treatment. *c-fos* antisense oligodeoxynucleotides (ODNs) were used as a tool to specifically study the impact of AP-1 on CORT-induced gene regulation in the dorsal hippocampus. The feasibility and specificity of the *c-fos* antisense approach has been demonstrated by a number of laboratories, including the present one (Sommer et al., 1993, 1996, 1998).

Materials and Methods

Animals and treatment. Male Sprague Dawley rats (Alab, Stockholm, Sweden), weighing 200–250 gm, were kept under a standard light/dark cycle (lights on at 6 A.M. and off at 6 P.M.) and constant room temperature (23°C) and had *ad libitum* access to tap water and food pellets.

Rats were bilaterally adrenalectomized under halothane anesthesia. The ADX model used has been characterized recently by Hansson et al. (2000). After surgery the rats were given 0.9% NaCl in the drinking water. Twenty-four hours after ADX rats were injected subcutaneously either with a single high dose of CORT (10 mg/kg, s.c.; ADX + CORT group; $n = 5$), which gives rise to full MR and GR occupation, or with a single injection of hormone vehicle (propylene glycol) alone (ADX + vehicle group; $n = 5$). All surgical procedures and hormone injections (injection volume 1 ml/kg) were performed between 7:00 and 9:00 A.M. Animals were anesthetized with sodium pentobarbital (Membumal; 100 mg/kg) 50 min and 2, 4, 8, and 24 hr after hormone or vehicle injection and were intracardially perfused with 100 ml ice-cold saline after blood samples for serum CORT had been collected by cardiac puncture. In another control experiment, non-injected and vehicle-injected ADX animals were killed after 50 min and 4 and 24 hr ($n = 4$ per time and treatment).

Using the same ADX animal model, a 15-mer partially phosphorothioated antisense ODN to *c-fos* (5'-GAA CAT CAT GGT CGT -3', 1 nmol in 1 μ l of Ringer's solution, as *c-fos* oligo group; $n = 6$) or a corresponding control random ODN (5'-GTA CCA ATC GGG ATT -3', as control oligo group; $n = 6$) was injected in a second experiment via a previously unilaterally implanted cannula guide into the dorsal hippocampus [1 μ l over 2 min; bregma anterior (A) –3.3 mm, lateral (L) +2.7 mm, ventral (V) –4.3 mm, according to the atlas of Paxinos and Watson (1982)]. Two hours later a single dose of CORT was given (10 mg/kg in propylene glycol, s.c.). After four hours the animals were anesthetized with sodium pentobarbital and intracardially perfused with 100 ml ice-cold saline after blood samples for serum CORT had been collected by cardiac puncture. After the rats were killed, brains were rapidly removed, frozen in liquid isopentane (–40°C), and stored at –70°C. Coronal sections (10 μ m) were taken at bregma levels close to the cannula guide and at bregma level –4 to –4.5 mm and mounted on slides (ProbeOn slides, Fischer/Biotech, Hampton, NH).

In a third experiment animals were unilaterally intrahippocampally injected with end-labeled [35 S]ATP antisense *c-fos* ODN (1 μ l over 1 min; bregma A –3.3 mm, L +2.7 mm, V –4.3 mm), and rats were killed by decapitation after 1 hr ($n = 4$) and 6 hr ($n = 4$). Transversal sections (10 μ m) were taken through the injection site, exposed for 2 and 7 d to β -Max Hyperfilm (Amersham Biosciences, Arlington Heights, IL), and counterstained with cresyl violet.

The animal experiments were approved by the local ethical committee (Stockholm Norra Försöksdjurs Etiska Kommittee).

Radioimmunoassay for corticosterone. Cardiac blood samples were collected in EDTA-containing tubes and centrifuged at 4 $\times$ g for 20 min at 4°C. Serum CORT levels were determined by radioimmunoassay (RIA) (Coat-a-count, Diagnostic Products Corporation, Los Angeles, CA). The RIA was performed with rat [125 I]CORT. The CORT assay had a detection limit of ~5.7 ng/ml.

RNA probe synthesis. The *bFGF* riboprobes, both antisense and sense,

were synthesized from a *SmaI*–*XhoI* fragment from rat cDNA (position: 525–1004 bp corresponding to the coding region for the mature protein) in Bluescript/SK⁺ (Stratagene, San Diego, CA). The *BDNF* riboprobes (antisense and sense) were generated from a 350 bp *PvuII*–*PstI* fragment from rat cDNA, coding for a corresponding protein, and subcloned into the Bluescript/SK⁺ plasmid. A 513 bp *EcoRI* fragment from the 5' end of the rat *MR* cDNA was subcloned into the vector pGEM4 (Promega, Madison, WI) and used for antisense and sense riboprobe generation. For the generation of the *GR* riboprobes (antisense and sense), we used a 673 bp *EcoRI*–*PstI* rat *GR* cDNA fragment (position: 1691–2364 bp, corresponding to the 3' portion of the coding region), subcloned into the vector pSP64 (Promega). The *c-fos* riboprobe was a 557 bp *BglII*–*StuI* fragment from rat cDNA in Bluescript KS II⁺ (Stratagene).

Antisense and sense RNA probes were synthesized from a 1 μ g linearized DNA template, incubated with transcription buffer (40 mM Tris-HCl, pH 7.5, 6 mM MgCl₂, 2 mM spermidine) in the presence of 12.5 nmol ATP, CTP, GTP, 500 pmol UTP, and 125 pmol [α - 35 S]UTP (1350 Ci/mmol; NEN/DuPont, Boston, MA), 1 U RNase inhibitor, and 1 U RNA polymerase (Roche Molecular Biochemicals, Bromma, Sweden) at 37°C for 1 hr. The DNA was removed by digestion with RNase-free DNase at 37°C for 15 min. The transcripts were purified using spin columns (Amersham Biosciences), and the quality of the riboprobes was analyzed on a 8 M urea/5% polyacrylamide gel (PAGE).

End-labeling of oligodeoxynucleotides. Radioactive labeling of phosphorothioate-modified antisense *c-fos* ODN was made by end-labeling with [35 S]ATP γ S and T4 polynucleotide kinase, followed by PAGE for purification. Aliquots of 5 $\times$ 10⁵ dpm were dried under vacuum and then mixed for injection with 1 nmol of cold ODN. The ODN mix was infused at 1 mM concentration in Ringer's solution.

In situ hybridization. The sections mounted on slides were brought to room temperature and fixed for 15 min in 4% paraformaldehyde in PBS, pH 7.0. After fixation the slides were washed in PBS, pH 7.4, for 10 min and rinsed two times in sterilized water for 5 min, each followed by a deproteinization of the tissue with 0.1 M HCl for 10 min. The slides were rinsed twice in PBS, pH 7.4, for 5 min and were acetylated in 0.1 M triethanolamine, pH 8.0/0.25% acetic anhydride for 20 min, washed again twice in PBS, pH 7.4, for 5 min, dehydrated in graded ethanol, and air dried. The slides were prehybridized in a humidified chamber with prehybridization buffer [50% deionized formamide, 50 mM Tris-HCl, pH 7.6, 25 mM EDTA, pH 8.0, 20 mM NaCl, 0.25 mg/ml yeast tRNA, 2.5 $\times$ Denhardt's solution (0.05% Ficoll, 0.05% polyvinylpyrrolidone, 0.05% bovine serum albumin)] at 37°C for 2–3 hr. After the prehybridization buffer was drained off the slides, the sections were hybridized with 80 μ l hybridization buffer (50% deionized formamide, 20 mM Tris-HCl, pH 7.6, 1 mM EDTA, pH 8.0, 0.3 M NaCl, 0.2 M DTT, 0.5 mg/ml yeast tRNA, 0.1 mg/ml poly-A-RNA, 1 $\times$ Denhardt's solution, 10% dextran sulfate) containing 0.5–1 $\times$ 10⁶ cpm of either the labeled antisense RNA or sense RNA. The sections were covered with siliconized coverslips and incubated at 44°C for 18 hr in a humidified chamber. After hybridization, the coverslips were removed by washing with 1 $\times$ standard saline citrate (SSC) at 48°C for 30 min, followed by washing twice in 0.5 $\times$ SSC/50% formamide at 48°C for 30 min. After two additional washings in 1 $\times$ SSC for 15 min at 48°C, the sections were treated with 1 μ g/ml RNAaseA in 0.5 M NaCl, 10 mM Tris-HCl, 5 mM EDTA, pH 8.0, for 45 min at 37°C. After two washing steps in 1 $\times$ SSC for 20 min at 55°C followed by a washing in 1 $\times$ SSC for 15 min at room temperature, the slides were dehydrated in graded ethanol and air dried. The hybridized sections were exposed for 1–4 weeks to β -Max Hyperfilm (Amersham Biosciences). A few sections of each animal from the second experiment were counterstained with cresyl violet for control of cannula guide placement.

Double labeling of *c-fos* transcripts and glial fibrillary acidic protein immunoreactivity. After *in situ* hybridization with S³⁵-labeled *c-fos* riboprobes, sections were rinsed in 0.1 M phosphate buffer, pH 7.4, and incubated with the primary antibody [glial fibrillary acidic protein (GFAP), monoclonal mouse anti-GFAP, 1:200; Chemicon International, Harrow, UK] for 48 hr at 4°C. The antibodies were diluted in 0.1 M phosphate buffer with 0.3% Triton X-100 (Sigma, St. Louis, MO), and all incubations were performed in humidified chambers. The sections were rinsed in 0.1 M phosphate buffer, and the secondary antibody (biotinyl-

ated horse anti-mouse, 1:100; Vector Laboratories, Burlingame, CA) was applied and incubated for 2 hr at room temperature. After rinsing the sections were incubated with solutions A and B in 0.1 M phosphate buffer (1:100; Vectastain ABC Reagent, Vector Laboratories) for 1 hr at room temperature. The sections were then exposed to 0.05 M Trizma buffer, pH 7.1, and reacted with 3,3'-diaminobenzidine tetrahydrochloride as a chromogen. The reaction was stopped by a pH shift in 0.05 M Trizma, pH 8.5. Sections were dehydrated in graded ethanol, coated with film emulsion (Ilford, Cheshire, UK), and stored in desiccated light-tight boxes at 4°C for 4 weeks. After development the sections were counterstained with cresyl violet and coverslipped with Mountex (Göteborgs Termomöterfabrik). Sections were digitized and evaluated using a Leica microscope connected with a high-resolution digital CCD video camera.

Double immunofluorescence staining. Sections were brought to room temperature, rehydrated, rinsed in 0.01 M phosphate buffer, and incubated with a mixture of two primary antibodies [polyclonal rabbit anti-*c-fos* antibody (Ab-5; Oncogene Research Products, San Diego, CA; 1:300) and either monoclonal mouse anti-GR antibody, a generous gift from Dr. A.-C. Wikström, Karolinska Institutet (Okret et al., 1984), 1:200, or a monoclonal mouse anti-neuronal nuclei antibody (NeuN; Chemicon, Temecula, CA; 1:200) or monoclonal mouse anti-GFAP (Chemicon; 1:200)] for 48–72 hr at 4°C. The sections were rinsed in 0.01 M phosphate buffer, and a mixture of the two secondary antibodies [biotinylated donkey anti-rabbit antibody (Amersham Biosciences; 1:50) and sheep anti-mouse-fluorescein isothiocyanate (FITC)-linked antibody (Amersham Biosciences; 1:200)] were added and incubated for 1–2 hr at room temperature. Sections were rinsed again and exposed to streptavidin–Texas Red (Amersham Biosciences; 1:100). Sections were rinsed briefly, mounted in a mounting medium for fluorescent signals (Dako, Carpinteria, CA), and coverslipped. The sections were evaluated by using a Zeiss Axiophot II microscope equipped with epilluminescence (mercury lamp) and filter sets (excitation/emission: FITC, 490/525 nm; Texas Red, 590/620 nm). The fluorescence microscope was connected to a high-resolution digital CCD video camera and a computer with OpenLab software.

Data analysis. Semiquantitative values of the mRNA levels were obtained by measuring the gray values of the film autoradiograms as illustrated in Figures 3 and 4 using an SAS Biovision image analyzing system (Avanzati, Milan, Italy). Radioactivity artifacts on autoradiograms close to tissue damage were carefully excluded in all measurements. Three measurements were performed for each region: (1) the total value, i.e., measurements of the region in the sections hybridized with [α -³⁵S]UTP-labeled antisense RNA; (2) the unspecific value, i.e., measurements of the corresponding region in the control sections hybridized with [α -³⁵S]UTP-labeled sense RNA; (3) the background value, i.e., measurements of the film background outside the sections. The transmittance percentage values (T%) of specific and unspecific labeling were obtained as described in Benfenati et al. (1986) and Zoli et al. (1991). From the T% values the optical density (O.D.) can be obtained ($O.D. = -\log T\%$). The data were expressed as optical density (means $\pm$ SEM). Two-way ANOVA for treatment and time followed by Fisher's PLSD *post hoc* test and Bonferroni's correction (Fisher's *p* value $\times$ number of brain regions analyzed; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$) was used for the statistical analysis in the time course experiment. One-way ANOVA followed by Bonferroni's correction (*p* value $\times$ number of brain regions analyzed; $*p < 0.05$; $**p < 0.01$) was used to analyze the treatment effect in the antisense *c-fos* experiment.

Results

Radioimmunoassay for corticosterone serum levels

After a single subcutaneous injection of CORT (10 mg/kg) to ADX rats, the serum levels of CORT were determined at the time of killing. Serum CORT levels were high after 50 min (2545 ± 321 ng CORT/ml blood serum; mean $\pm$ SEM; $n = 5$) and then gradually declined over the 2 hr (805 ± 143 ng CORT/ml; $n = 5$), 4 hr (147 ± 27 ng CORT/ml; $n = 5$), and 8 hr time interval (29 ± 4 ng CORT/ml; $n = 5$) down to undetectable serum CORT levels 24 hr

after the injection. In all vehicle-injected ADX rats, serum levels of CORT were undetectable in each time interval analyzed.

In the second experiment, CORT (10 mg/kg, s.c.) was injected 2 hr after antisense *c-fos* or control ODN treatment in 24 hr ADX rats. Four hours later, serum CORT levels did not differ between the *c-fos* oligo group (275 ± 80 ng CORT/ml serum) and the control oligo animal group (372 ± 65 ng CORT/ml serum; one-way ANOVA: $F_{(1,10)} = 0.89$; not significant).

Effects of corticosterone treatment on gene expression in the dorsal hippocampus

In situ hybridization analysis was performed for *bFGF*, *BDNF*, *GR*, *MR*, and *c-fos* mRNAs. Optical density values from the dorsal hippocampus were obtained from autoradiograms, and the data were analyzed either by two-way ANOVA in the CORT time course experiment or by one-way ANOVA in the *c-fos* experiment, followed by Bonferroni's correction. The optical density data and the results of the *post hoc* analysis are summarized in Tables 1–3. To better demonstrate the most important actions of CORT on gene expression levels, the optical density values were transformed into relative changes (%) in relation to the ADX + vehicle-treated group in Figure 1. Figure 3*d* shows the effects of CORT on gene expression levels after *c-fos* blockade as relative changes (percentage) in relation to control ODN-injected ADX rats. The distribution patterns for *bFGF*, *BDNF*, *GR*, and *MR* mRNA levels in the dorsal hippocampus are shown in Figure 2 and are similar to those described in Hansson et al. (2000). *In situ* hybridization of *c-fos* mRNA showed a moderate and low expression pattern in CA1 to CA4 and DG, respectively (see Fig. 2).

Time course of gene expression after corticosterone treatment

bFGF

bFGF mRNA levels were significantly and time-dependently increased after acute CORT treatment in all hippocampal subregions as shown by two-way ANOVA for treatment (CA1: $F_{(1,40)} = 132.3$, $p < 0.0001$; CA2: $F_{(1,40)} = 70.1$, $p < 0.0001$; CA3: $F_{(1,40)} = 148.9$, $p < 0.0001$; DG: $F_{(1,40)} = 123.6$, $p < 0.0001$) and interaction of treatment and time (CA1: $F_{(1,40)} = 18.7$, $p < 0.0001$; CA2: $F_{(1,40)} = 7.7$, $p = 0.0001$; CA3: $F_{(1,40)} = 28.6$, $p < 0.0001$; DG: $F_{(1,40)} = 46$, $p < 0.0001$). Bonferroni's corrected *post hoc* analysis revealed that CORT induced strong and significant increases of *bFGF* mRNA levels in all hippocampal subfields between 2 and 8 hr after treatment (CA1: 90 to 119%, CA2: 110 to 146%, CA3: 85 to 157%, DG: 84 to 204%) (Table 1, Figs. 1, 2). Only in CA2 did CORT induce long-lasting changes of *bFGF* mRNA levels observed even after 24 hr (105% increase) (Table 1). Levels of *bFGF* mRNAs were not changed in vehicle-treated ADX rats over the time course of the experiment in all analyzed subregions.

BDNF

CORT induced time-dependent significant changes of *BDNF* mRNA levels as revealed by two-way ANOVA for treatment (CA1: $F_{(1,39)} = 38$, $p < 0.0001$; CA3: $F_{(1,39)} = 12.1$, $p = 0.0013$; DG: $F_{(1,39)} = 108$, $p < 0.0001$) and interaction of treatment and time (CA1: $F_{(1,39)} = 2.9$, $p = 0.03$; CA3: $F_{(1,39)} = 0.8$, not significant; DG: $F_{(1,39)} = 14.7$, $p < 0.0001$). The corrected *post hoc* test suggested a CORT-induced decrease of *BDNF* mRNA levels after 2–8 hr treatment in CA1 (30 to 53%) and DG (44 to 67%) (Table 1, Figs. 1, 2). In CA3, levels of *BDNF* mRNA were only significantly decreased after 2 hr (26%) (Table 1, Fig. 1). *BDNF* mRNA

Table 1. Effects of corticosterone (10 mg/kg, s.c.) on gene expression levels in the dorsal hippocampus of ADX rats

	Time	CA1	CA2	CA3	DG
<i>bFGF</i> mRNA					
ADX + vehicle	50 min	125 ± 4	405 ± 101	148 ± 8	150 ± 4
ADX + CORT	50 min	140 ± 6	340 ± 21	144 ± 13	164 ± 9
ADX + vehicle	2 hr	119 ± 5	281 ± 14	130 ± 5	153 ± 4
ADX + CORT	2 hr	226 ± 9***	652 ± 52***	240 ± 13***	336 ± 17***
ADX + vehicle	4 hr	125 ± 16	390 ± 62	118 ± 9	149 ± 10
ADX + CORT	4 hr	274 ± 18***	818 ± 48***	303 ± 16***	453 ± 28***
ADX + vehicle	8 hr	95 ± 4	361 ± 115	107 ± 11	130 ± 7
ADX + CORT	8 hr	205 ± 17***	888 ± 14***	269 ± 20***	239 ± 23***
ADX + vehicle	24 hr	117 ± 4	257 ± 12	126 ± 1	157 ± 3
ADX + CORT	24 hr	119 ± 5	528 ± 13**	126 ± 10	130 ± 3
<i>BDNF</i> mRNA					
ADX + vehicle	50 min	158 ± 12	n.e.	600 ± 44	331 ± 33
ADX + CORT	50 min	144 ± 15	n.e.	588 ± 31	284 ± 17
ADX + vehicle	2 hr	141 ± 7	n.e.	666 ± 42	317 ± 20
ADX + CORT	2 hr	98 ± 14*	n.e.	490 ± 51*	138 ± 10***
ADX + vehicle	4 hr	133 ± 3	n.e.	592 ± 46	340 ± 17
ADX + CORT	4 hr	63 ± 5***	n.e.	500 ± 31	112 ± 13***
ADX + vehicle	8 hr	136 ± 15	n.e.	545 ± 49	362 ± 23
ADX + CORT	8 hr	79 ± 3***	n.e.	434 ± 25	203 ± 14***
ADX + vehicle	24 hr	138 ± 12	n.e.	599 ± 68	251 ± 7
ADX + CORT	24 hr	122 ± 8	n.e.	493 ± 42	261 ± 15
<i>GR</i> mRNA					
ADX + vehicle	50 min	359 ± 13	373 ± 28	107 ± 5	211 ± 8
ADX + CORT	50 min	324 ± 25	316 ± 10	106 ± 9	186 ± 15
ADX + vehicle	2 hr	379 ± 18	353 ± 18	121 ± 7	230 ± 13
ADX + CORT	2 hr	179 ± 14***	166 ± 23***	63 ± 9***	87 ± 1***
ADX + vehicle	4 hr	343 ± 21	288 ± 15	104 ± 14	230 ± 13
ADX + CORT	4 hr	122 ± 5***	104 ± 10***	39 ± 12***	109 ± 5***
ADX + vehicle	8 hr	341 ± 41	318 ± 40	128 ± 16	236 ± 15
ADX + CORT	8 hr	164 ± 6***	159 ± 9***	48 ± 6***	167 ± 9***
ADX + vehicle	24 hr	409 ± 12	367 ± 13	128 ± 9	248 ± 15
ADX + CORT	24 hr	266 ± 16***	249 ± 21***	77 ± 5**	192 ± 7**
<i>MR</i> mRNA					
ADX + vehicle	50 min	496 ± 37	848 ± 61	496 ± 40	414 ± 25
ADX + CORT	50 min	538 ± 31	940 ± 69	602 ± 9*	451 ± 18
ADX + vehicle	2 hr	523 ± 23	944 ± 54	551 ± 29	420 ± 9
ADX + CORT	2 hr	384 ± 32*	747 ± 46	459 ± 21	352 ± 24
ADX + vehicle	4 hr	407 ± 16	769 ± 17	466 ± 34	379 ± 6
ADX + CORT	4 hr	249 ± 36**	607 ± 87	306 ± 35***	168 ± 15***
ADX + vehicle	8 hr	405 ± 31	728 ± 51	549 ± 69	363 ± 27
ADX + CORT	8 hr	295 ± 19	550 ± 23	335 ± 27***	250 ± 21***
ADX + vehicle	24 hr	455 ± 45	809 ± 54	606 ± 48	382 ± 18
ADX + CORT	24 hr	359 ± 23	701 ± 34	343 ± 25***	345 ± 9
<i>c-fos</i> mRNA					
ADX + vehicle	50 min	107 ± 10	n.e.	134 ± 7	92 ± 5
ADX + CORT	50 min	85 ± 10	n.e.	120 ± 12	57 ± 7*
ADX + vehicle	2 hr	41 ± 4	n.e.	102 ± 6	56 ± 4
ADX + CORT	2 hr	31 ± 7	n.e.	78 ± 8	30 ± 3*
ADX + vehicle	4 hr	39 ± 8	n.e.	62 ± 8	40 ± 5
ADX + CORT	4 hr	24 ± 3	n.e.	56 ± 4	34 ± 3
ADX + vehicle	8 hr	47 ± 7	n.e.	69 ± 14	45 ± 7
ADX + CORT	8 hr	50 ± 6	n.e.	69 ± 3	42 ± 6
ADX + vehicle	24 hr	48 ± 3	n.e.	82 ± 14	48 ± 6
ADX + CORT	24 hr	48 ± 7	n.e.	73 ± 8	35 ± 6

>Data are expressed as optical densities ($\times 10^3$, means $\pm$ SEM); n.e. = not evaluated. Analysis at Bregma levels -3 to -4 mm were performed by two-way ANOVA (treatment $\times$ time) followed by Fisher's PLSD test and Bonferroni's correction. Significances between mRNA levels are shown between corticosterone-treated (ADX + CORT) and hormone vehicle-treated (ADX + vehicle) ADX rats at the same time point. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; $n = 5$ per group.

levels were not changed in vehicle-treated ADX rats during the time course in all subregions analyzed.

GR

A significant time-dependent decrease of *GR* mRNA levels by CORT was found in all hippocampal subregions using two-way ANOVA for treatment (CA1: $F_{(1,39)} = 149.2$, $p < 0.0001$; CA2:

$F_{(1,39)} = 116.2$, $p < 0.0001$; CA3: $F_{(1,39)} = 62.7$, $p < 0.0001$; DG: $F_{(1,39)} = 130.6$, $p < 0.0001$) and interaction of treatment and time (CA1: $F_{(1,39)} = 6.2$, $p = 0.0006$; CA2: $F_{(1,39)} = 3.3$, $p = 0.0194$; CA3: $F_{(1,39)} = 4.0$, $p = 0.0085$; DG: $F_{(1,39)} = 8.8$, $p < 0.0001$). Corrected *post hoc* analysis revealed strong and significant long-lasting decreases of *GR* mRNA levels from 2 to 24 hr (CA1: 35 to

Figure 1. Densitometric evaluation of *in situ* hybridization from autoradiograms. Values are given in percentage of the ADX group (mean $\pm$ SEM; $n = 5$) versus vehicle-treated control ADX rats. Statistical analysis was performed by two-way ANOVA (treatment $\times$ time) followed by Fisher's PLSD test and Bonferroni's correction. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus ADX group. CA1, CA3, Cornu Ammon areas; DG, dentate gyrus. For details on treatment, see Materials and Methods.

64%; CA2: 32 to 64%; CA3: 40 to 63%; DG: 23 to 62%) (Table 1, Figs. 1, 2). *GR* mRNA levels were not changed in vehicle-treated rats at any time point analyzed.

MR

The expression of *MR* was significantly and time-dependently decreased by CORT in all hippocampal subregions as demonstrated by two-way ANOVA for treatment (CA1: $F_{(1,40)} = 21.8$, $p < 0.0001$; CA2: $F_{(1,40)} = 10.8$, $p = 0.0021$; CA3: $F_{(1,38)} = 47.3$, $p < 0.0001$; DG: $F_{(1,38)} = 42.9$, $p < 0.0001$) and interaction of treatment and time (CA1: $F_{(1,40)} = 3.2$, $p = 0.023$; CA3: $F_{(1,38)} = 12.8$, $p < 0.0001$; DG: $F_{(1,38)} = 12.2$, $p < 0.0001$). Bonferroni's corrected Fisher's *post hoc* analysis revealed a significant decrease in *MR* mRNA levels after 2 and 4 hr in CA1 (27 to 39%) (Table 1, Figs. 1, 2), after 4 hr in CA3 (34 to 43%) (Table 1, Figs. 1, 2), and between 4 and 8 hr in DG (31 to 56%) (Table 1, Figs. 1, 2). Interestingly, there was a small and significant increase in *MR* mRNA levels by 21% in CA3 after 50 min (Table 1, Fig. 1). In CA2, the corrected *post hoc* test did not show significant differences between CORT- and vehicle-treated rats (Table 1, Figs. 1, 2). Furthermore, *MR* mRNA levels were not changed in vehicle-treated rats at any time point analyzed.

c-fos

The expression of *c-fos* was time-dependently and significantly decreased after CORT treatment only in the DG as suggested by two-way ANOVA for treatment ($F_{(1,38)} = 21.8$, $p < 0.0001$) and interaction of treatment and time ($F_{(1,38)} = 3.3$, $p = 0.021$). The corrected *post hoc* test showed significant decreases of *c-fos* mRNA levels in the DG between 50 min and 2 hr (38 to 46%) (Table 1, Figs. 1, 2). There was no treatment or interaction effect in other hippocampal regions.

Furthermore, the two-way ANOVA demonstrated a strong time effect in all subregions (CA1: $F_{(1,38)} = 33$, $p < 0.0001$; CA3: $F_{(1,40)} = 16.6$, $p < 0.0001$; DG: $F_{(1,38)} = 16.1$, $p < 0.0001$). Corrected *post hoc* analysis revealed strong and significant increases

in *c-fos* mRNA levels at 50 min in both vehicle- and CORT-treated rats compared with the other time points in CA1 (2–24 hr: 123 to 245% increase), CA3 (4–24 hr: 63 to 116% increase), and DG (2–24 hr: 64 to 130% increase) (Table 1).

There was a decrease in *c-fos* mRNA levels by 27% in vehicle injected versus non-injected ADX rats at 50 min in DG (two-way ANOVA for treatment and time: $F_{(2,14)} = 6.0$, $p < 0.0133$) (Table 2). There were no significant differences in *c-fos* mRNA levels between injected and non-injected ADX rats in CA1 and CA3 at any time interval (Table 2).

Effects of intrahippocampal *c-fos* antisense ODN injection on corticosterone-induced gene expression

bFGF

In CA1, *c-fos* antisense ODN increased *bFGF* mRNA levels (44%) 4 hr after CORT treatment compared with control ODN-treated rats as revealed by one-way ANOVA followed by Bonferroni's correction ($F_{(1,11)} = 22.4$, corrected $p = 0.0024$) (Table 3, Fig. 3*c,d*). *bFGF* mRNA levels were not affected by antisense treatment

in other subregions. The antisense effect was observed only at bregma levels close to the injection site.

BDNF

Blockade of *c-fos* expression enhanced the 4 hr CORT response on *BDNF* in CA1 by decreasing mRNA levels to 38% versus control oligo-treated rats (one-way ANOVA: $F_{(1,7)} = 32.7$, corrected $p = 0.0021$) (Table 3, Fig. 3*c,d*). Other hippocampal subregions were not affected by antisense treatment. The antisense effect was observed only at bregma levels close to the injection site.

GR and MR

Intrahippocampal *c-fos* blockade did not affect the CORT response on *GR* and *MR* mRNA levels in any subregion (Table 3).

c-fos

Antisense *c-fos* treatment induced a strong increase of *c-fos* mRNA levels in CA1 (89%) as shown by one-way ANOVA followed by Bonferroni's correction ($F_{(1,10)} = 10.8$, corrected $p = 0.0246$) (Table 3, Fig. 3*d*). The increase of *c-fos* mRNA levels was observed only at bregma levels close to the injection site.

Distribution of end-labeled antisense *c-fos* oligodeoxynucleotides

The distribution of [35 S]ATP end-labeled phosphorothioate modified antisense *c-fos* ODN after intrahippocampal injection was demonstrated by autoradiography. A high intensity labeling was found close to the injection site 1 hr after injection (Fig. 3*b*). After 6 hr, weak labeling was found with the same distribution as seen after 1 hr (data not shown).

Cellular distribution of *c-fos* mRNA in the hippocampus

Double labeling of *c-fos* mRNA and GFAP immunoreactivity (–ir) demonstrates that *c-fos* is mainly located outside of GFAP-immunoreactive profiles in large and weakly cresyl violet-stained

Figure 2. Dark-field microphotographs from autoradiograms of *in situ* hybridization of *bFGF*, *BDNF*, *GR*, and *MR* mRNA levels 4 hr after injection and of *c-fos* mRNA levels 50 min after subcutaneous injection in ADX + vehicle-treated (left panel) and ADX + CORT-treated (10 mg/kg, s.c.) rats. Arrowheads show mRNA signals in the dentate gyrus. Bregma levels were -3 to -4 mm. Scale bar, 1 mm.

Table 2. Effects of injection stress on *c-fos* mRNA levels in the dorsal hippocampus of ADX rats

Treatment	Time	CA1	CA3	DG
ADX	50 min	74 ± 5	89 ± 5	67 ± 1
ADX + vehicle	50 min	61 ± 2	87 ± 7	49 ± 10*
ADX	4 hr	42 ± 6	41 ± 3	41 ± 1
ADX + vehicle	4 hr	45 ± 3	49 ± 4	47 ± 1
DX	24 hr	36 ± 6	43 ± 4	31 ± 4
ADX + vehicle	24 hr	41 ± 7	63 ± 7	42 ± 4

Data are expressed as optical densities ($\times 10^3$, means $\pm$ SEM); n.e. = not evaluated. Analysis at Bregma levels -3 to -4 mm were performed by two-way ANOVA (treatment $\times$ time) followed by Fisher's PLSD test and Bonferroni's correction. Significances between mRNA levels are shown between hormone vehicle-injected (ADX + vehicle) and non-injected ADX rats at the same time point. * $p < 0.05$; $n = 4$ per group.

cell nuclei within the pyramidal cell layer of CA and the granular cell layer of DG, probably representing nerve cells. The distribution of the *c-fos* signal appears in a "speckled" pattern, i.e., a few large neurons here and there are strongly labeled above the average *c-fos* signal (Fig. 4).

Colocalization of *c-fos* and GR, *c-fos* and NeuN, and *c-fos* and GFAP-ir in the hippocampus

The colocalization of *c-fos*-ir and GR-ir and markers for neurons (NeuN-ir) and astrocytes (GFAP-ir) were studied in CA1, CA3, and DG of ADX rats 1.5 hr after corticosterone treatment. Double immunofluorescence analysis of nuclear *c-fos*-ir (red color) and nuclear GR-ir or nuclear NeuN-ir (both with green color)

Table 3. Effects of *c-fos* antisense oligonucleotides on gene expression in response to corticosterone (10 mg/kg, s.c.) in the dorsal hippocampus of ADX rats

I. Analysis at Bregma levels close to the implanted cannula guide

	CA1	CA2	CA3	DG
bFGF mRNA				
Control oligo	102 ± 7	347 ± 16	99 ± 8	214 ± 11
Antisense <i>c-fos</i> oligo	147 ± 7**	428 ± 28	103 ± 11	229 ± 15
BDNF mRNA				
Control oligo	29 ± 1	n.e.	108 ± 9	127 ± 17
Antisense <i>c-fos</i> oligo	18 ± 1**	n.e.	118 ± 7	116 ± 15
GR mRNA				
Control oligo	238 ± 12	234 ± 14	121 ± 6	279 ± 27
Antisense <i>c-fos</i> oligo	303 ± 26	308 ± 37	152 ± 9	335 ± 30
MR mRNA				
Control oligo	388 ± 28	860 ± 41	534 ± 47	473 ± 29
Antisense <i>c-fos</i> oligo	446 ± 35	828 ± 49	557 ± 38	544 ± 58
<i>c-fos</i> mRNA				
Control oligo	53 ± 5	n.e.	78 ± 11	185 ± 26
Antisense <i>c-fos</i> oligo	100 ± 13*	n.e.	131 ± 16	177 ± 17

II. Analysis at Bregma levels -4 to -4.5 mm

	CA1	CA2	CA3	DG
bFGF mRNA				
Control oligo	107 ± 6	319 ± 24	91 ± 7	207 ± 17
Antisense <i>c-fos</i> oligo	129 ± 14	386 ± 36	96 ± 10	215 ± 22
BDNF mRNA				
Control oligo	28 ± 3	n.e.	81 ± 17	108 ± 30
Antisense <i>c-fos</i> oligo	15 ± 3	n.e.	103 ± 8	99 ± 23
GR mRNA				
Control oligo	300 ± 14	259 ± 14	148 ± 7	328 ± 26
Antisense <i>c-fos</i> oligo	269 ± 13	236 ± 19	131 ± 13	291 ± 26
MR mRNA				
Control oligo	408 ± 17	874 ± 43	507 ± 36	483 ± 22
Antisense <i>c-fos</i> oligo	351 ± 29	744 ± 66	470 ± 61	463 ± 47
<i>c-fos</i> mRNA				
Control oligo	62 ± 12	n.e.	80 ± 17	208 ± 29
Antisense <i>c-fos</i> oligo	92 ± 15	n.e.	128 ± 23	160 ± 26

Data are expressed as optical densities ($\times 10^3$, means $\pm$ SEM); n.e. = not evaluated. Analysis was performed by one-way ANOVA followed by Fisher's PLSD test and Bonferroni's correction. Significances between mRNA levels are shown between *c-fos* antisense oligodeoxynucleotides (antisense *c-fos* oligo) and randomized oligodeoxynucleotides (control oligo) treated animal groups for each gene analyzed; * $p < 0.05$; ** $p < 0.01$; $n = 6$ per group.

showed a predominant colocalization (yellow color) of the relatively low number of *c-fos*-ir nuclei, with GR-ir and NeuN-ir nuclei found in large numbers in all analyzed regions of the hippocampus (Fig. 5). There was no colocalization of *c-fos*-ir and GFAP-ir in the hippocampus. Thus, *c-fos*-ir is found mainly in GR-ir neurons of the hippocampus; however, only a small population of the GR-ir neurons is *c-fos*-ir.

Discussion

The data presented here support the view that hippocampal neurotrophic factors and corticosteroid receptors are target genes for glucocorticoid regulation (Chao et al., 1989, 1998; Herman et al., 1989; Pfeiffer et al., 1991; Barbany and Persson, 1992; Chadi et al., 1993; Follesa and Mochetti, 1993; Herman, 1993; Chao and McEwen, 1994; Riva et al., 1995; Smith et al., 1995a,b; Mochetti et al., 1996; Schaaf et al., 1997, 1998, 2000; Hansson et al., 2000, 2001; Molteni et al., 2001). A time course for CORT revealed peak hormone responses for these genes, except for *c-fos*, at the 4 hr time interval. Hippocampal *bFGF* mRNA levels are strongly and positively regulated by CORT, in contrast to the negative regulation of *BDNF*, *GR*, and *MR* mRNA levels by CORT. *c-fos* mRNA levels are negatively regulated exclusively in the DG. Using the same ADX animal model and time intervals for hormonal peak

Figure 3. *a*, Schematic representation to show the position of the cannula guide and of the sampled areas for densitometric evaluation in a coronal section through the dorsal hippocampus (bregma level -3.3 mm). CA1–CA3, Cornu Ammon areas; DG, dentate gyrus. *b*, Distribution of ³⁵S-end-labeled antisense *c-fos* ODN 1 hr after intrahippocampal injection as shown in a cresyl violet-counterstained coronal section (bregma level -3 to -4 mm). *c*, Dark-field microphotographs from *in situ* hybridization of autoradiograms of *bFGF* and *BDNF* mRNA levels in response to CORT (10 mg/kg, s.c.) after randomized (left panel) and antisense *c-fos* ODN (right panel) treatment. Arrowheads show mRNA signals in the CA1 region (bregma levels -3 to -4.5 mm). Scale bar, 1 mm. *d*, Densitometric evaluation of *in situ* hybridization autoradiograms. Values are given as percentage of random oligo-treated group (mean ± SEM; $n = 6$). Statistical analysis was performed by one-way ANOVA (treatment) followed by Bonferroni's correction. * $p < 0.05$, ** $p < 0.01$ versus random oligo group. CA1, Cornu Ammon area. For details on treatment, see Materials and Methods.

response, the main finding obtained on the basis of the antisense approach was that *c-fos* can suppress the CORT-induced responses in *bFGF* and *BDNF* expression in CA1, demonstrating an antagonistic interaction of activated GR/MR with *c-fos* mechanisms in the control of these genes.

Effects of corticosterone on hippocampal *c-fos* gene expression

In the present paper, *c-fos* mRNA levels are significantly down-regulated by CORT in the DG. Previous communications on CORT effects on *c-fos* expression reported conflicting results. In tissue cultures, glucocorticoids increased *c-fos* mRNA levels, as shown for AtT-20 pituitary tumor cells (Lin et al., 1992) and primary hypothalamic cells (Luo and Jackson, 1998), decreased *c-fos* mRNA levels in C6 glioma cells (Yin and Howells, 1992), or

Figure 4. Bright-field photomicrographs showing a strongly labeled nerve cell enriched in silver grains, representing *c-fos* transcripts, and a GFAP-ir (brown color) astroglial cell lacking silver grains in the same cresyl violet counterstained section in the CA1 region of corticosterone-treated (10 mg/kg, s.c.; 4 hr) ADX rat. CA1, Cornu Ammon area. For details on treatment, see Materials and Methods. Scale bar, 15 μ m. Bregma level -3 to -4.5 mm.

had no effect in AtT-20 cells (Autelitano, 1994). Chronic treatment with the synthetic glucocorticoid dexamethasone inhibits stress-induced *c-fos* expression in the hypothalamic paraventricular nucleus (Kovács and Sawchenko, 1996), which is in line with the present results.

Normally, basal *c-fos* levels in naive animals are low (Herdgen and Leah, 1998). Here, significantly increased *c-fos* mRNA levels in all experimental groups were observed at the 50 min time point compared with the other time points. Handling of the animals (Asanuma et al., 1992) or the novel environment (Wirtshafter et al., 1998), or both, may be involved in the initial induction of *c-fos* mRNA levels reported in the present paper, and in fact the impact of such effects for hippocampal *c-fos* expression has been reported previously (Kovács, 1998). Except for the DG, the present *c-fos* induction neither seems to be dependent on circulating glucocorticoids (hormone- vs vehicle-injected ADX rats) nor does it appear to be associated with the injection stress (non-injected vs vehicle-injected ADX rats). A dissociation of *c-fos* upregulation from the hormonal stress response was also found by others (Melia et al., 1994; Helmreich et al., 1996; Ryabinin et al., 1999). In DG the injection procedure by itself appears to reduce *c-fos* levels. The opposing effect on *c-fos* expression by novelty and pain stimuli may be a result of different pathways involved in their mediation. These effects, however, can clearly be separated from the CORT effect. Furthermore, the expression of neurotrophic factor and corticosteroid receptor mRNA levels shows a stable baseline over time in control rats. Their CORT-induced gene regulation therefore may not be confounded by the initial rise in *c-fos* mRNA levels.

As stated above, CORT seems to counteract the initial increase of *c-fos* expression in the DG. The mechanism involved may be that activated GR/MR can suppress *c-fos* transcription via bind-

ing to a putative GRE in the promoter region (Wang and Howells, 1994) or that activated GR/MR can interact with Jun/Fos heterodimers leading to reduced AP-1 activity (Teurich and Angel, 1995).

Effects of antisense blockade of *c-fos* on gene expression

Inducible transcription factors may not only interact directly with DNA but may also indirectly modify transcription processes by complexing with other transcriptionally operating proteins. Therefore the second experiment tested whether AP-1, i.e. *c-fos*, is involved in the CORT regulation of genes by use of intrahippocampal antisense “knockdown” of *c-fos* expression. Antisense treatment increased *bFGF* and decreased *BDNF* mRNA levels after CORT administration, and hence an enhancement of the CORT-induced transcriptional regulation of these genes was found. The CA1 area contains fewer *bFGF* and *BDNF* mRNA signals than the CA3 or the DG, and the magnitude of changes induced by the antisense treatment was modest using semiquantitative measurements. Nevertheless, such a change in a modest signal may have consequences for the relatively low signaling at the receptors for these neurotrophic factors in CA1, because the corresponding changes in the proteins may be at least as large. An inhibitory effect of *c-fos* on gene regulation by glucocorticoids has earlier been described in tissue culture studies by Diamond et al. (1990), Jonat et al. (1990), Lucibello et al. (1990), and Pearce and Yamamoto (1993), but to our knowledge this is the first demonstration that this effect is also present *in vivo*. *c-fos* antisense treatment did not affect the autoregulation of *GR* and *MR* by CORT. This is in line with recent findings that in NIH3T3 cells *c-fos* under basal, low-level conditions is not the limiting step for *c-jun*-mediated *GR* promoter repression (Cabral et al., 2001). In contrast, *c-fos* overexpression seems to enhance *GR* promoter activity (Wei and Vedeckis, 1997). The findings that *c-fos/c-jun* heterodimers but not *c-jun* homodimers bind to the AP-1-like site in the *GR* promoter emphasize the complexity of *GR* promoter regulation even further (Cabral et al., 2001).

The injection of ODNs into various regions of the brain has been used in a large number of studies to investigate the role of *c-fos* in the regulation of downstream genes or in behavioral or physiological responses associated with the activation of *c-fos* (for review, see Szklarczyk and Kaczmarek, 1999). For example, a number of papers have demonstrated that infusions of *c-fos* antisense ODNs into the striatum can suppress central stimulant-induced motor behavior and the associated expression of *c-fos* (Dragunow et al., 1993; Heilig et al., 1993; Sommer et al., 1993). Antisense blockade of *c-fos* in the hippocampus attenuated excitatory amino acid neurotoxicity (Lu et al., 1997) and seizure-induced heat shock protein hsp70 expression (Baille et al., 1997) and demonstrated a role of *c-fos* in learning and memory (Grimm et al., 1997; Tischmeyer and Grimm, 1999).

Along with these and other studies, the *c-fos* antisense approach has been validated by various control experiments to demonstrate specificity and to establish the pharmacokinetics (Szklarczyk and Kaczmarek, 1999). For antisense ODNs targeted around the translational start site of the *c-fos* mRNA (used also in the present study), efficacy and specificity have been demonstrated at the level of translation (reduction of *c-fos* protein between 70 and 90%) (Sommer et al., 1993; Grimm et al., 1997; Hebb and Robertson, 1997a,b; Lu et al., 1997; Lofti and Armelin,

Figure 5. Fluorescence photomicrographs involving double immunolabeling procedures showing the colocalization (yellow color) of *c-fos*-ir (Texas Red) with NeuN-ir (FITC, green, left) or with GR-ir (FITC, green, right), but not with GFAP-ir (FITC, middle) in the CA1 region of corticosterone-treated (10 mg/kg, s.c.; 1.5 hr) ADX rats. CA1, Cornu Ammon area. For details on treatment, see Materials and Methods. Scale bar, 40 μ m. Bregma level = -3 to -4.5 mm.

1998; Zhang et al., 1999) and in the reduction of AP-1 complexes (Liu et al., 1994; Xu et al., 2001). Furthermore, the pharmacokinetics of *c-fos* antisense ODN in the brain has been analyzed systematically (Hebb and Robertson, 1997a,b; Grzanna et al., 1998; Sommer et al., 1998). The optimal period of antisense efficacy varied between 1 and 10 hr, depending on the degree of phosphorothioate substitution (for end-capped and fully phosphorothioated ODN, respectively) (Sommer et al., 2000). Neuronal uptake and activity for the partially phosphorothioated ODN used in the present study was found from 90 min to >4 hr (Sommer et al., 1996, 1998), giving support for the antisense action observed under the present conditions, that is, 6 hr after the administration of the antisense *c-fos* ODN and 4 hr after the CORT challenge. It should be noted that the antisense action studied close to the damaged area was always compared with the action of control ODNs in the same region to exclude unspecific actions.

In addition to the antisense effects on *bFGF* and *BDNF* expression in the CA1 region, a strong upregulation was also found of the *c-fos* transcript itself in this region. Interestingly, in previous studies increased levels of *c-fos* mRNA was observed after intrastriatal *c-fos* antisense treatment (Sommer et al., 1996). These findings are not unexpected in light of the strong feedback regulation of *c-fos* transcription via AP-1 (Sassone-Corsi et al., 1988; Schonthal et al., 1989).

Both the regulation of *c-fos* target genes (i.e., *bFGF*, *BDNF*) and the *c-fos* autoregulation appear to be region specific, because in the present study it is observed only in the CA1 region but not in the adjacent DG, although the ODN can be found in both regions (Fig. 3*b*). The absence of an antisense effect in the DG may be explained by the fact that CORT is already suppressing *c-fos* expression in this region in contrast to CA1.

To demonstrate anatomic specificity of the intrahippocampally injected ODNs, we used radiolabeled partially phosphorothioated ODNs that have been found to remain intact in brain tissue up to 24 hr after injection (Sommer et al., 2000). The distribution of these ODNs was restricted to the vicinity of the injection site and appears to match the observed antisense activity. Similarly, others reported that intrahippocampally injected ODNs do not spread outside of the hippocampus and remain within ~ 1 mm³ of the injected area (Baille et al., 1997; Grimm et al., 1997).

Despite the demonstrated antagonistic functional interaction between GR and *c-fos*, only a small subpopulation of neurons within the pyramidal cell layer of the CA1 region is *c-fos*-ir; the majority of these nerve cells are also GR-ir. No *c-fos*-ir is found in astroglial cells, demonstrating a major involvement of neuronal

cells. Nevertheless, it seems possible that the actions of the antisense *c-fos* treatment on *bFGF* and *BDNF* gene expression found in large numbers of nerve and glial cells all over the CA1 area could be related to an antagonistic interaction between GR and *c-fos* in this small population of nerve cells. Thus, this antagonistic interaction at the promoter regions of such genes and others in this special group of cells could lead to a marked alteration in their neuronal activity, whether representing glutamate or GABA neurons, which may initiate a cascade of events leading to an altered control of the majority of *BDNF* nerve cells and *bFGF* glial cells in this region, involving recurrent collaterals and widespread terminal networks. In this way it becomes possible to understand how a potential interaction of GR and *c-fos* in a small group of neurons could lead to the demonstrated GR-enhancing actions of antisense *c-fos* treatment on *BDNF* and *bFGF* expression in neuronal and glial networks, respectively, the majority of which exhibit no *c-fos*-ir but only GR-ir. Thus, the response of these cells to GR activation with regard to *BDNF* and *bFGF* expression becomes altered in view of their altered afferent input, triggered by the altered antagonistic interactions of GR and *c-fos* on the antisense treatment in this small population of nerve cells found mainly in the CA1 pyramidal cell layer. It must be noted, however, that in the damaged area not analyzed for *c-fos*-ir, *c-fos*-ir could exist in cells not containing GR-ir. Therefore, actions of antisense *c-fos* treatment may exist that occur independently of GRES.

In summary, the time course for CORT in the ADX rat revealed a peak hormone effect on neurotrophic factors (*bFGF*, *BDNF*) and corticosteroid receptor (*GR* and *MR*) mRNA levels at the 4 hr time interval. *c-fos* mRNA levels were negatively regulated in the dentate gyrus at the 50 min to 2 hr time interval. The major finding was that local antisense *c-fos* ODN treatment significantly and selectively enhanced the CORT action only in the CA1 region on the expression of *bFGF* and *BDNF* mRNA versus randomized control ODN treatment. Thus, it seems as if within the CA1 area a tonic *c-fos* mechanism exists that reduces GR- or MR-induced effects, or both, on gene expression of *bFGF* and *BDNF*.

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