

Glucocorticoids Stimulate Inflammatory 5-Lipoxygenase Gene Expression and Protein Translocation in the Brain

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Abstract: In the brain, the expression of 5-lipoxygenase (5-LO), the enzyme responsible for the synthesis of inflammatory leukotrienes, increases during aging. Anti-inflammatory drugs are currently being evaluated for the treatment of aging-associated neurodegenerative diseases such as Alzheimer's disease. Although generally considered antiinflammatory, glucocorticoids, whose production also increases during aging, are not particularly effective in this disease. In human monocytes, 5-LO mRNA content increases on exposure to the synthetic glucocorticoid dexamethasone, which prompted us to hypothesize that glucocorticoids might increase 5-LO expression in the brain as well. We treated rats for 10 days either with corticosterone (implanted subcutaneously) or with dexamethasone (injected daily); they were killed on day 10 after pellet implantation or 24 h after the 10th dexamethasone injection. We found increased levels of 5-LO mRNA and protein in hippocampus and cerebellum of glucocorticoid-treated rats; 5-LO-activating protein (FLAP) mRNA content was not affected. Using western immunoblotting, we also observed the concurrent translocation of 5-LO protein from cytosol to membrane, an indication of its activation. Thus, glucocorticoid-mediated up-regulation of the neuronal 5-LO pathway may contribute to rendering an aging brain vulnerable to degeneration. **Key Words:** 5-Lipoxygenase—FLAP (5-lipoxygenase-activating protein)—Corticosterone—Dexamethasone—Hippocampus—Cerebellum. *J. Neurochem.* **73**, 693–699 (1999).

5-Lipoxygenase (5-LO), the enzyme crucial for the biosynthesis of inflammatory eicosanoids, the leukotrienes, is present in neurons (Lammers et al., 1996), and its expression in the rat brain increases during aging (Uz et al., 1998) or after surgical removal of the pineal gland (pinealectomy) (Uz et al., 1997). In the brain, leukotriene synthesis (Simmet and Tippler, 1991) and 5-LO mRNA content and/or 5-LO protein redistribution/translocation (Ohtsuki et al., 1995; Manev et al., 1998) also increase during seizures or after stroke. Protection of the brain from injury/excitotoxicity has been achieved with anti-inflammatory 5-LO inhibitors (Baskaya et al., 1996; Uz et al., 1998). Thus, increased 5-LO expression in the

brain has been associated with its increased vulnerability to injury (Uz et al., 1997, 1998; Manev et al., 1998). It also appears that the neurodegeneration associated with the human immunodeficiency virus may involve induction of the 5-LO gene (Maccarrone et al., 1998). Also, aside from their putative role in neurodegeneration, eicosanoids may exert various other, still poorly understood, effects on neuronal transmission (Piomelli, 1994; Christie et al., 1999).

It is believed that for full enzymatic activity, 5-LO requires a cofactor, the protein termed 5-LO-activating protein (FLAP). At least two concepts have been proposed to explain how FLAP activates 5-LO: One theorizes that FLAP is a docking protein for 5-LO, and the other proposes that FLAP is an arachidonate transfer protein that binds free arachidonate and presents it to 5-LO (for review, see Peters-Golden, 1998). Thus, the translocation of cytosolic 5-LO to different particulate compartments, including the membrane-bound FLAP, is an important mechanism associated with 5-LO activation (Lepley et al., 1996). Both 5-LO and FLAP are expressed in the brain, and their colocalization was demonstrated in CA1 pyramidal neurons of the hippocampus (Lammers et al., 1996). In a gerbil model of transient forebrain ischemia with reperfusion, it was observed that 5-LO translocated from cytosolic to particulate/membrane fractions within 3 min during reperfusion and that this translocation was associated with increased leukotriene production (Ohtsuki et al., 1995).

Recent characterization of the 5-LO gene promoter indicated that this gene could be regulated by several hormones (Hoshiko et al., 1990). Included in the sequence of the response element of the 5-LO promoter is also a partial glucocorticoid response element, which

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Abbreviations used: cyc, cyclophilin; FLAP, 5-lipoxygenase-activating protein; 5-LO, 5-lipoxygenase; TBST buffer, 10 mM Tris base, 0.15 M NaCl, and 0.05% Tween 20.

suggests that 5-LO expression might be affected by glucocorticoids as well. In vitro, the expression of 5-LO was found to be stimulated by a synthetic glucocorticoid, dexamethasone (Riddick et al., 1997). Because levels of glucocorticoids (corticosterone in rats and cortisol in humans) are elevated under conditions that also elevate those of brain 5-LO mRNA, e.g., aging or pinealectomy (Landfield et al., 1978; Oxenkrug et al., 1984; Lupien et al., 1998), and because they are known to be capable of promoting neurodegeneration (Sapolsky and Pulsinelli, 1985), we hypothesized that glucocorticoids might stimulate the expression and/or translocation of 5-LO in the brain in vivo. We tested and confirmed this hypothesis in experiments with rats.

MATERIALS AND METHODS

Animals and drug treatment

Male Sprague-Dawley rats (weighing 200–225 g; Taconic Farms) were housed under a 12-h light–dark cycle (darkness commenced at 18:00 h) with free access to food/water. Corticosterone pellets (25 or 50 mg, releasable over 10 days at rates of 1.19 and 2.38 mg/day, respectively) and corresponding placebo pellets were purchased from Innovative Research of America (Sarasota, FL, U.S.A.). They were implanted subcutaneously. Dexamethasone was suspended in sesame oil and injected daily (1 mg/kg, s.c.). The experimental protocol was approved by the Institutional Animal Care and Use Committee. Rats were killed on day 10 after pellet implantation or 24 h after the 10th dexamethasone injection. Brains were immediately removed, dissected, and kept at -80°C until analyzed. Plasma was collected, and corticosterone was quantified by a radioimmunoassay (Diagnostic Products Co., Los Angeles, CA, U.S.A.). It was not detectable in the plasma of dexamethasone-treated rats, but corticosterone levels were 205 and 365% of those of controls in rats treated with 25- and 50-mg corticosterone pellets, respectively (data not shown).

RT-PCR analysis of 5-LO/cyclophilin (cyc) and FLAP/cyc mRNA ratios

We used two approaches to assay quantitatively the content of 5-LO or FLAP mRNA in brain samples: (a) the initial screening assay, in which we calculated the ratios between 5-LO or FLAP and the cyc RT-PCR products; and (b) the quantitative assay of absolute mRNA content with specific internal standards.

Because cyc is a constitutive gene, its expression can be used as a control when inducible genes are studied. To allow the coamplification of 5-LO and FLAP mRNAs with the more abundant cyc mRNA, pilot studies were conducted to determine the optimal relative primer concentrations and cycle number, whereby the PCR was still within the exponential phase of amplification for all transcripts. Specific 5-LO and cyc amplification primers were designed and prepared as described elsewhere (Uz et al., 1997); the FLAP primers were designed to allow amplification of 113–496 bp of the FLAP mRNA (Dixon et al., 1990).

The total RNA (1 μg) isolated from hippocampal or cerebellar samples was denatured at 80°C for 6 min and then reverse-transcribed with cloned Moloney murine leukemia virus reverse transcriptase (200 U; GibcoBRL, Chagrin Falls, OH, U.S.A.) in RT buffer containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl_2 , and 1 mM deoxynucleotide triphos-

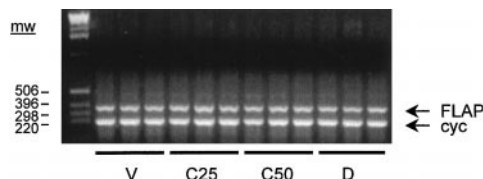


FIG. 1. RT-PCR assay of FLAP/cyc mRNA ratios. Shown is a typical gel obtained after RT-PCR assay of hippocampal samples using both FLAP (upper band) and cyc (lower band) primers. V, vehicle; C25, corticosterone, 25-mg pellet; C50, corticosterone, 50-mg pellet; D, dexamethasone. Shown are three samples per group. No differences among groups were observed. For the results of quantitative analyses with this assay, see Table 1.

phates (GibcoBRL) using random hexamers (5 mM; Pharmacia Biotech, Piscataway, NJ, U.S.A.) and ribonuclease inhibitor (28 U; Amersham, Arlington Heights, IL, U.S.A.) in a volume of 20 μl . The RT mixture was incubated at 37°C for 60 min to promote cDNA synthesis. The reaction was terminated by heating the samples at 98°C for 5 min, and the mixture was quick-chilled on ice. As a control, in all assays one RT was performed in the absence of RNA.

After termination of the RT, cDNA aliquots containing reverse-transcribed material were amplified with Hot *Tub* DNA polymerase (Amersham) in a thermal cycler (model 9600; Perkin-Elmer, Oak Brook, IL, U.S.A.). The amplification mixture contained cDNA, 0.5 μM 5-LO- or FLAP- and 0.15 μM cyc-specific primers, 200 μM deoxynucleotide triphosphates, 1.5 mM MgCl_2 , 50 mM Tris-HCl (pH 9.0), 20 mM ammonium sulfate, 15 mM KCl, and 1.5 U of Hot *Tub* polymerase in a 100- μl volume. Trace amounts of [^{32}P]dCTP (0.5 μCi per sample; Amersham) were included during the PCR step for subsequent quantification. The PCR mixture was amplified for 30 cycles with denaturation (94°C , 15 s), annealing (60°C , 30 s), and elongation (72°C , 30 s) amplification steps. The reaction was terminated with a 5-min final elongation step, and products were separated by agarose gel electrophoresis. To quantify the amount of the product corresponding to the amplified mRNA, the ethidium bromide-stained bands (for an example, see Fig. 1) were excised, and the radioactivity was determined by Cerenkov counting. The results (ratios) are presented in arbitrary units.

Quantitative 5-LO and cyc RT-PCR mRNA assay with internal standards

Internal standard templates for 5-LO and cyc were generated by site-directed mutagenesis using PCR overlap extension, as previously described (Uz et al., 1997, 1998). Decreasing concentrations of either 5-LO or cyc internal standard cRNA were added to 1 μg of the total RNA isolated from hippocampal or cerebellar samples. The RNA/cRNA mixtures were denatured at 80°C for 6 min and then reverse-transcribed as described above and reported elsewhere (Uz et al., 1997, 1998). Following amplification, aliquots were digested with *Bgl*III in triplicate and assayed by agarose gel electrophoresis. The ethidium bromide-stained bands (Fig. 2) were excised, and the radioactivity was determined by Cerenkov counting. The results of RT-PCR were calculated in attomoles of specific RNA, i.e., 5-LO or cyc, per microgram of total RNA and are presented as a percentage of the vehicle-treated control.

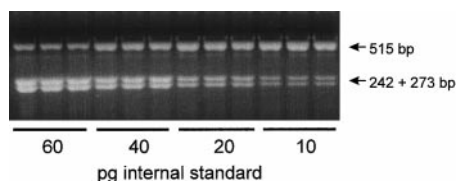


FIG. 2. Gel electrophoresis of the PCR products obtained using the 5-LO-specific primers and internal standards. Shown are the ethidium bromide-stained bands obtained after the assay of the hippocampal RNA in the presence of decreasing concentrations of internal standards. The smaller bands (242 and 273 bp) are the cleaved products of the internal standard RNA (for details, see Materials and Methods).

5-LO western immunoblotting

The hippocampus and the cerebellum were dissected out and homogenized in homogenizing buffer, containing 20 mM Tris-HCl, 2 mM EGTA, 5 mM EDTA, 1.5 mM pepstatin, 2 mM leupeptin, 0.5 mM phenylmethylsulfonyl fluoride, 0.2 units/ml aprotinin, and 2 mM dithiothreitol, using a Polytron. The supernatant was centrifuged at 100,000 *g* for 60 min at 4°C. The resulting supernatant was a portion of the cytosol (S1) fraction, and the pellet was resuspended in the homogenizing buffer and centrifuged again at 100,000 *g* for 60 min at 4°C. The resulting supernatant (S2) was combined with the S1 fraction, and the combination was used for analyses as the "cytosol" fraction. The remaining pellet was homogenized in homogenizing buffer and was used in analyses as the "membrane" fraction. The concentration of protein in these two fractions was determined using the procedure of Lowry et al. (1951).

Equal volumes of protein samples (20–40 μ g of protein; cytosol or membrane) and gel loading solution [50 mM Tris-HCl (pH 6.8), 4% β -mercaptoethanol, 1% sodium dodecyl sulfate, 40% glycerol, and a trace amount of bromophenol blue] were mixed, and the samples were boiled for 3 min and kept on ice for 10 min. The samples were loaded onto 7.5% (wt/vol) acrylamide gel using the Mini Protean II gel apparatus (Bio-Rad, Hercules, CA, U.S.A.). The gels were electrophoresed using 25 mM Tris base, 192 mM glycine, and 0.1% (wt/vol) sodium dodecyl sulfate at 150 V. The proteins were subsequently transferred electrophoretically to an ECL nitrocellulose membrane (Amersham) using the Mini TransBlot transfer unit (Bio-Rad) at 150 mA constant current. Membranes were washed with TBST buffer (10 mM Tris base, 0.15 M NaCl, and 0.05% Tween 20) for 10 min.

The blots were blocked by incubating them with 5% (wt/vol) powdered nonfat milk in TBST buffer, 2 ml of Nonidet P-40, and 0.02% (wt/vol) sodium dodecyl sulfate (pH 8.0). Thereaf-

ter, the blots were incubated overnight with the primary anti-5-LO antibody (rabbit polyclonal; Cayman Chemical, Ann Arbor, MI, U.S.A.) at a dilution of 1:1,000. The blots were then washed with TBST buffer, incubated with horseradish peroxidase-linked secondary antibody (anti-rabbit IgG; 1:1,000) for 4 h at room temperature, and processed with the Amersham ECL kit; blots were then washed with TBST and exposed to ECL film. Before starting the immunolabeling, the procedure was standardized using 5–200 μ g protein of the membrane or cytosol fraction. We found that the optical density of the bands varied linearly with concentrations up to 160 μ g of protein. To normalize our data, we simultaneously measured β -actin immunoreactivity using the monoclonal primary antibody (Sigma, St. Louis, MO, U.S.A.; 1:3,000 for 2 h) and anti-mouse IgG (1:3,000 for 2 h) as the secondary antibody. The optical densities of the bands on the autoradiograms were quantified using the Loats (Westminster, MD, U.S.A.) Image Analysis System, and the optical density of the 5-LO band (78 kDa) was corrected by the optical density of the corresponding β -actin band (46 kDa). The values are expressed as a percent of the control.

Statistics

Data are given as mean \pm SEM values. Statistical analysis of results was performed using ANOVA followed by the Mann-Whitney *U* test or by the Kruskal-Wallis one-way ANOVA on ranks; *p* < 0.05 was accepted as significant.

RESULTS

Effect of glucocorticoids on 5-LO and FLAP mRNAs

In the present study, we used RT-PCR assays of 5-LO/*cyc* and FLAP/*cyc* mRNA ratios as the first step in investigating the action of glucocorticoids on 5-LO and/or FLAP expression. Because *cyc* is a constitutive gene, its expression can be used as a control when inducible genes are studied. For example, when hippocampal 5-LO mRNA content was increased owing to pinealectomy, no changes occurred in the levels of *cyc* mRNA (Uz et al., 1997). Table 1 shows the results obtained from hippocampal samples; neither corticosterone nor dexamethasone exerted any effects on the FLAP/*cyc* ratio (Fig. 1 and Table 1), whereas they both increased the 5-LO/*cyc* ratio (Table 1). [Similar results were observed in the cerebellum (data not shown).] In rat brain, 5-LO mRNA has been localized using the *in situ* hybridization technique; the most intense 5-LO mRNA signal was found in hippocampus and cerebellum, fol-

TABLE 1. Effect of glucocorticoids on FLAP/*cyc* and 5-LO/*cyc* mRNA ratios in rat hippocampus

	Vehicle	Corticosterone		
		25-mg pellet	50-mg pellet	Dexamethasone
FLAP/ <i>cyc</i>	1.98 \pm 0.03	1.78 \pm 0.06	1.65 \pm 0.09	1.90 \pm 0.17
5-LO/ <i>cyc</i>	1.63 \pm 0.19	2.02 \pm 0.06	2.44 \pm 0.12 ^a	2.58 \pm 0.21 ^a

Data are mean \pm SEM values.

^a *p* < 0.05 compared with the vehicle-treated group by Kruskal-Wallis test (*n* = 6).

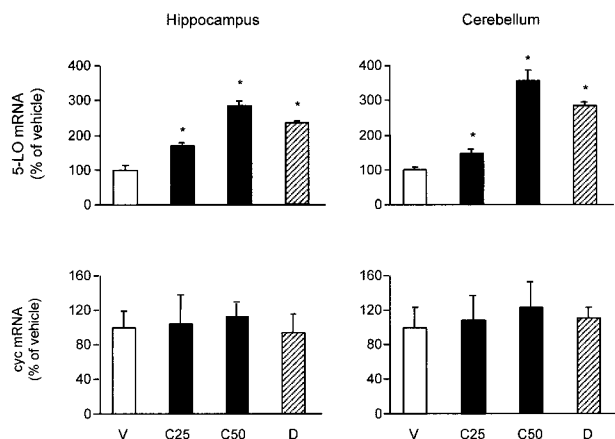


FIG. 3. Stimulatory effect of glucocorticoid treatment on hippocampal and cerebellar 5-LO mRNA content assayed using quantitative RT-PCR with internal standards (Uz et al., 1997, 1998). Systemic administration for 10 days of corticosterone (25- or 50-mg pellets; C25 and C50, respectively) or its synthetic analogue, dexamethasone (D), induced an increase (relative to vehicle-treated controls; V) in level of 5-LO mRNA but not in the content of mRNA encoding a structural protein, cyc, in rat hippocampus and cerebellum. Data are mean \pm SEM (bars) values ($n = 5$ per group), expressed as a percent of corresponding control absolute mRNA content: (**upper left**) 5-LO hippocampus, 116 ± 16 amol/ μ g of total RNA; (**upper right**) 5-LO cerebellum, 92 ± 6.7 amol/ μ g; (**lower left**) cyc hippocampus, 488 ± 90 amol/ μ g; and (**lower right**) cyc cerebellum, 495 ± 114 amol/ μ g. * $p < 0.05$ in comparison with the respective control by ANOVA followed by Mann-Whitney U test.

lowed by primary olfactory cortex, neocortex, thalamus, hypothalamus, and brainstem (Lammers et al., 1996). These authors also noted prominent colocalization of 5-LO and FLAP mRNAs in hippocampal and cerebellar neurons, i.e., in the regions in which we observed the stimulatory action of glucocorticoids on the 5-LO/cyc mRNA ratio.

Because these data indicated that glucocorticoids affected 5-LO but not FLAP mRNA, we investigated more thoroughly the effects of these hormones on 5-LO expression using quantitative RT-PCR with internal standards (Uz et al., 1997, 1998). Again, we found increased hippocampal 5-LO mRNA content after prolonged hyperglucocorticoidemia, i.e., after 10 days of continuous treatment with the glucocorticoid corticosterone or dexa-

methasone (Fig. 3, upper left). Moreover, we observed a similar increase in 5-LO mRNA content caused by glucocorticoids in the cerebellum, the brain region with the next highest, after the hippocampus, basal 5-LO mRNA levels (Fig. 3, upper right). We also used quantitative RT-PCR with specific internal standards to assay the amounts of cyc mRNAs (Fig. 3, lower left and right); glucocorticoids selectively increased 5-LO mRNA levels without affecting cyc mRNA content (Fig. 3).

Effect of glucocorticoids on 5-LO protein content and translocation

Corticosterone and dexamethasone were effective not only in increasing 5-LO mRNA content, but also in increasing the amount of total, i.e., cytosol plus membrane, 5-LO-immunoreactive protein in both hippocampus and cerebellum (Table 2). A concomitant increase in both 5-LO mRNA and 5-LO protein levels is indicative that glucocorticoids increase 5-LO gene expression.

The translocation of brain 5-LO from cytosol to membrane is considered to be an indication of 5-LO activation (Ohtsuki et al., 1995). Hence, we assayed the effects of glucocorticoids on the cytosol/membrane distribution of 5-LO protein (Fig. 4). Quantitative analyses of these experiments are shown in Fig. 4. Compared with results with vehicle-treated controls, both corticosterone and dexamethasone treatments decreased 5-LO protein content in cytosol while increasing the content of 5-LO protein in the corresponding membrane fractions (Fig. 5).

DISCUSSION

Taken together, our data on the glucocorticoid-induced increase in hippocampal and cerebellar content of 5-LO mRNA and 5-LO-immunoreactive protein indicate that glucocorticoids stimulate 5-LO gene expression. Several lines of evidence suggest that 5-LO is indeed subject to transcriptional regulation by hormones. For example, the pineal hormone melatonin was reported to exert an inhibitory effect on the 5-LO gene promoter in human B lymphocytes (Steinhilber et al., 1995), whereas stress hormones, such as glucocorticoids, stimulate 5-LO gene expression in human monocytes and THP-1 cells (Riddick et al., 1997).

TABLE 2. Effect of glucocorticoids on total (cytosol + membrane) 5-LO protein content in rat hippocampus and cerebellum (expressed as 5-LO/ β -actin ratios)

	Vehicle	Corticosterone		Dexamethasone
		25-mg pellet	50-mg pellet	
Hippocampus	2.45 ± 0.03	2.61 ± 0.07^a	2.70 ± 0.07^a	2.52 ± 0.10
Cerebellum	2.93 ± 0.07	3.23 ± 0.05^a	3.53 ± 0.06^a	3.34 ± 0.05^a

Data are mean \pm SEM values.

^a $p < 0.01$ compared with the vehicle-treated group by Kruskal-Wallis test ($n = 6$).

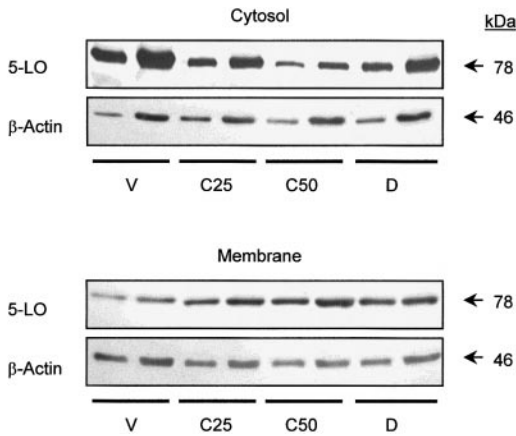


FIG. 4. Western immunoblot with 5-LO and β -actin antibodies of hippocampal cytosol and membrane fractions and effect of glucocorticoids. Samples from rats treated with vehicle (V), corticosterone [25- (C25) or 50-mg (C50) pellets], or dexamethasone (D) were processed in two concentrations (20 μ g, left lane; 40 μ g, right lane). Note a decrease in the cytosol fraction and an increase in the membrane fraction of a glucocorticoid-treated animal. The results of quantitative analysis of 5-LO/ β -actin ratios are shown in Fig. 5.

Thus, this earlier work suggests that an up-regulation of 5-LO gene expression would occur in conditions of melatonin deficiency and/or in conditions of hyperglucocorticoidemia. Both these hormonal changes, i.e., decreased melatonin and elevated glucocorticoid levels, are prevalent in elderly subjects and have been related to brain aging (Magri et al., 1997). Whereas a stimulatory effect of melatonin deficiency on hippocampal 5-LO mRNA was observed in experiments with pinealectomy (Uz et al., 1997), our present results demonstrate for the first time that elevated glucocorticoid levels lead to increased 5-LO mRNA and protein content in the brain *in vivo*.

In the brain, it has been observed that activation of 5-LO, i.e., increased leukotriene production, is associated with the translocation of 5-LO-immunoreactive protein from cytosol to membrane (Ohtsuki et al., 1995). We have found that glucocorticoids also induce 5-LO translocation from cytosol to membrane. Although we did not observe a concomitant change in FLAP mRNA content nor did we measure leukotriene production, the fact that we found FLAP expressed in brain samples in which 5-LO was up-regulated suggests that all components of the 5-LO pathway required for its functioning and activation are present in rat hippocampus and cerebellum. Previous work by others has described more specifically the colocalization of 5-LO and FLAP in these brain areas (Lammers et al., 1996). Whether the brain FLAP expression *in vivo* is regulated differently from its expression in the blood cells *in vitro* should be further investigated. In particular, Riddick et al. (1997) reported that both 5-LO and FLAP expression is increased after *in vitro* dexa-

methasone treatment of blood monocytes or THP-1 cells in culture.

Recently, it was demonstrated in neuronal cultures *in vitro* that up-regulation of 5-LO gene expression may lead to cell death, presumably by increasing lipid peroxidation (Maccarone et al., 1998). Glucocorticoids, which we found to be capable of up-regulating 5-LO expression, also increase neuronal vulnerability to degeneration. Most of the neurodamaging effects of glucocorticoids are usually observed in the hippocampus (Lupien et al., 1998); however, glucocorticoids increase the severity of ischemic neuronal damage not only in this brain area but also in numerous other regions (Sapolsky and Pulsinelli, 1985). This suggests that a general glucocorticoid-sensitive mechanism is operative in increased neuronal vulnerability. The increase in glucocorticoid levels appears to be enhanced in pathologies such as senile dementia (Magri et al., 1997). Moreover, in a recent study Lupien et al. (1998) reported that aged humans with prolonged elevated levels of cortisol exhibit reduced hippocampal volume and deficits in hippocampus-dependent memory tasks compared with normal-cortisol controls. Increased aging-associated vulnerability of the hippocampus to excitotoxic injury has recently been associated with up-regulation in 5-LO gene expres-

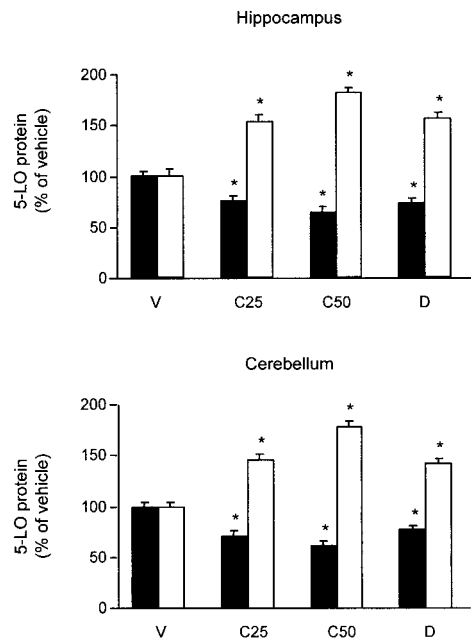


FIG. 5. Glucocorticoids induce 5-LO translocation from cytosol (solid columns) to membrane (open columns) in rat hippocampus and cerebellum. V, vehicle; C25, corticosterone 25-mg pellet; C50, corticosterone 50-mg pellet; D, dexamethasone. Blots (as shown in Fig. 4) were measured densitometrically, and the 5-LO/ β -actin ratios of samples obtained from six rats per group were statistically analyzed. Data are mean \pm SEM (bars) values, expressed as a percent of the corresponding vehicle-treated groups (defined as 100%). * $p < 0.05$ in comparison with the corresponding vehicle-treated group by Kruskal-Wallis test.

sion (Uz et al., 1998). The results of our present study are consistent with the possibility that 5-LO up-regulation could be one of the mediators of the adverse action of glucocorticoids on neuronal viability.

Several mechanisms have been considered as possible mediators of glucocorticoid-induced neuronal damage, including that of signaling via specific glucocorticoid receptors. Although the 5-LO promoter has been implicated in the inhibitory action of melatonin on 5-LO gene expression (Steinhilber et al., 1995), presumably due to an action of melatonin via the putative nuclear melatonin receptors (Wiesenberg et al., 1998), data that point to a direct action of glucocorticoid receptors at the 5-LO promoter are lacking. Such a possibility is indicated, however, by the presence in the response element of the 5-LO promoter [bp -1,804 to -1,809 (Hoshiko et al., 1990)] of the sequence "AGAACA," which is indicative of the partial glucocorticoid response consensus element (Luisi et al., 1991; Funder, 1997). Nevertheless, further studies are needed to investigate the functional relevance of this element in the 5-LO promoter.

Although our observation that prolonged elevation of glucocorticoid levels in vivo is capable of up-regulating the inflammatory 5-LO pathway in the brain appears to be at odds with the conventional view that glucocorticoids are antiinflammatory hormones that decrease the synthesis of various inflammatory molecules, this discrepancy has already been noted (Riddick et al., 1997). The growing body of evidence that argues against the general view that the role of glucocorticoids in the regulation of inflammatory and immune responses is only inhibitory has recently been reviewed (Wiegers and Reul, 1998). Moreover, it has been observed that, in contrast to antiinflammatory drugs, which exert a beneficial effect in Alzheimer's patients, glucocorticoids are less effective (Breitner, 1996).

Our previous work (Manev et al., 1996, 1998; Uz et al., 1997, 1998) and the results of this study suggest that an enhancement in the 5-LO inflammatory pathway in the brain might be triggered by hormonal alterations, most of which are associated with aging. Thus, it is warranted that antiinflammatory strategies targeted to this pathway should be seriously considered for their possible role in altering the pathobiology of glucocorticoid/stress-associated neurodegenerations, as well as in aging-associated neurodegenerative diseases such as Alzheimer's disease (Manev, 1999).

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REFERENCES

- Baskaya M. F., Hu M. D. Y., Donaldson D., Maley M., Rao A. M., Prasad M. R., and Dempsey R. J. (1996) Protective effect of the 5-lipoxygenase inhibitor AA-861 on cerebral edema after transient ischemia. *J. Neurosurg.* **85**, 112–116.
- Breitner J. C. S. (1996) Inflammatory processes and antiinflammatory drugs in Alzheimer's disease: a current appraisal. *Neurobiol. Aging* **17**, 789–794.
- Christie M. J., Vaughan C. W., and Ingram S. L. (1999) Opioids, NSAIDs and 5-lipoxygenase inhibitors act synergistically in brain via arachidonic acid metabolism. *Inflamm. Res.* **48**, 1–4.
- Dixon R. A. F., Diehl R. E., Opas E., Rands E., Vickers P. J., Evans J. F., Gillard J. W., and Miller D. K. (1990) Requirement of a 5-lipoxygenase-activating protein for leukotriene synthesis. *Nature* **343**, 282–284.
- Funder J. W. (1997) Glucocorticoid and mineralocorticoid receptors: biology and clinical relevance. *Annu. Rev. Med.* **48**, 231–240.
- Hoshiko S., Radmar O., and Samuelsson B. (1990) Characterization of the human 5-lipoxygenase gene promoter. *Proc. Natl. Acad. Sci. USA* **87**, 9073–9077.
- Lammers C.-H., Schweitzer P., Facchinetti P., Arrang J.-M., Madamba S. G., Siggins G. R., and Piomelli D. (1996) Arachidonate 5-lipoxygenase and its activating protein: prominent hippocampal expression and role in somatostatin signaling. *J. Neurochem.* **66**, 147–152.
- Landfield P. W., Waymire J. C., and Lynch G. (1978) Hippocampal aging and adrenocorticoids: quantitative correlations. *Science* **202**, 1098–1102.
- Lepley R. A., Muskardin D. T., and Fitzpatrick F. L. (1996) Tyrosine kinase activity modulates catalysis and translocation of cellular 5-lipoxygenase. *J. Biol. Chem.* **271**, 6179–6184.
- Lowry O. H., Rosebrough N. J., Farr A. L., and Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.
- Luisi B. F., Xu W. X., Otwinowski Z., Freedman L. P., Yamamoto K. R., and Sigler P. B. (1991) Crystallographic analysis of the interaction of the glucocorticoid receptor with DNA. *Nature* **352**, 497–505.
- Lupien S. J., deLeon M., deSanti S., Convit A., Tarshish C., Nair N. P. V., Thakur M., McEwen B. S., Hauger R. L., and Meaney M. J. (1998) Cortisol levels during human aging predict hippocampal atrophy and memory deficits. *Nat. Neurosci.* **1**, 69–73.
- Maccarrone M., Navarra M., Corasanti M. T., Nistico G., and Finazzi Agro A. (1998) Cytotoxic effect of HIV-1 coat glycoprotein gp120 on human neuroblastoma CHP100 cells involves activation of arachidonate cascade. *Biochem. J.* **333**, 45–49.
- Magri F., Locatelli M., Balza G., Molla G., Cuzzoni G., Fioravanti M., Solerte S. B., and Ferrari E. (1997) Changes in endocrine circadian rhythms as markers of physiological and pathological brain aging. *Chronobiol. Int.* **14**, 385–396.
- Manev H. (1999) 5-Lipoxygenase gene promoter polymorphism and onset of Alzheimer's disease. *Med. Hypotheses* (in press).
- Manev H., Uz T., Kharlamov A., and Joo J.-Y. (1996) Increased brain damage after stroke or excitotoxic seizures in melatonin-deficient rats. *FASEB J.* **10**, 1546–1551.
- Manev H., Uz T., and Qu T. (1998) Early upregulation of hippocampal 5-lipoxygenase following systemic administration of kainate to rats. *Restor. Neurol. Neurosci.* **12**, 81–85.
- Ohtsuki T., Matsumoto M., Hayashi Y., Yamamoto K., Kitagawa K., Ogawa S., Yamamoto S., and Kamada T. (1995) Reperfusion induces 5-lipoxygenase translocation and leukotriene C4 production in the ischemic brain. *Am. J. Physiol.* **268**, H1249–H1257.
- Oxenkrug G. F., McIntyre I. M., and Gershon S. (1984) Effects of pinealectomy and aging on the serum corticosterone circadian rhythm in rats. *J. Pineal Res.* **1**, 181–185.
- Peters-Golden M. (1998) Cell biology of the 5-lipoxygenase pathway. *Am. J. Respir. Crit. Care Med.* **157** (Suppl.), S227–S232.
- Piomelli D. (1994) Eicosanoids in synaptic transmission. *Crit. Rev. Neurobiol.* **8**, 65–83.
- Riddick C. A., Ring W. L., Baker J. R., Hodulik C. R., and Bigby T. D. (1997) Dexamethasone increases expression of 5-lipoxygenase and its activating protein in human monocytes and THP-1 cells. *Eur. J. Biochem.* **246**, 112–118.
- Sapolsky R. M. and Pulsinelli W. (1985) Glucocorticoids potentiate ischemic injury to neurons: therapeutic implications. *Science* **229**, 1397–1400.

- Simmet T. and Tippler B. (1991) On the relation between cerebral cysteinyl-leukotriene formation and epileptic seizures. *Brain Res.* **540**, 283–286.
- Steinhilber D., Brungs M., Werz O., Wiesenberg I., Danielsson C., Kahlen J.-P., Nayeri S., Schröder M., and Carlberg C. (1995) The nuclear receptor for melatonin represses 5-lipoxygenase gene expression in human B lymphocytes. *J. Biol. Chem.* **270**, 7037–7040.
- Uz T., Longone P., and Manev H. (1997) Increased hippocampal 5-lipoxygenase mRNA content in melatonin-deficient, pinealectomized rats. *J. Neurochem.* **69**, 2220–2223.
- Uz T., Pesold C., Longone P., and Manev H. (1998) Aging-associated up-regulation of neuronal 5-lipoxygenase expression: putative role in neuronal vulnerability. *FASEB J.* **12**, 439–449.
- Wieggers G. J. and Reul J. M. H. M. (1998) Induction of cytokine receptors by glucocorticoids: functional and pathological significance. *Trends Pharmacol. Sci.* **19**, 317–321.
- Wiesenberg I., Missbach M., and Carlberg C. (1998) The potential role of the transcription factor RZR/ROR as a mediator of nuclear melatonin signaling. *Restor. Neurol. Neurosci.* **12**, 143–150.