Glucocorticoid receptors are required for up-regulation of neuronal 5-lipoxygenase (5LOX) expression by dexamethasone[1]

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SPECIFIC AIM

>5-lipoxygenase (5LOX) is the key enzyme in the synthesis of inflammatory leukotrienes (LTs) from arachidonic acid. We had previously found that hyperglucocorticoidemia or dexamethasone treatment increase 5LOX expression in the rat brain in vivo. The aim of this study was to characterize in detail the stimulatory action of dexamethasone on 5LOX expression in neural cultures in vitro.

PRINCIPAL FINDINGS

1. Dexamethasone increases 5LOX mRNA and protein content in primary cultures of rat cerebellar granule neurons (CGN) in a concentration- and time-dependent manner

Exposure of CGN to dexamethasone increased the content of both 5LOX mRNA (assayed by quantitative RT-PCR) and 5LOX protein (Western blotting). These increases were concentration dependent and became statistically significant with 100 nM dexamethasone. Time course studies revealed that dexamethasone treatment increased the content of 5LOX mRNA and protein as early as 3 h and that these increases persisted for at least 24 h of dexamethasone treatment (**Fig. 1**).

2. Dexamethasone increases the capacity of CGN to produce cysteinyl leukotrienes (cysLTs)

The capacity of CGN to produce LTs was assayed by enzyme-linked assay, i.e., by determination of cell-free supernatants for the predominant 5LOX products LTB_4 and cysLTs (i.e., LTC_4, LTD_4, and LTE_4). Cultures were treated with 1 μM dexamethasone for 24 h (controls were treated with a vehicle for the same period of time). In the last 15 min of treatment, cultures were supplemented with 10 μM arachidonic acid (substrate for LT synthesis) and 2 μM A23187 (calcium ionophore; calcium is required for full 5LOX enzymatic activity). In the absence of added arachidonic acid, we did not detect LTs in the culture medium even if these cultures were treated with A23187. After adding arachidonic acid and A23187, we readily detected cycLTs but not LTB_4 (the lower limit of detection was 13 pg/ml) and the content of cysLTs was greater in cultures pretreated with dexamethasone than in the corresponding controls: vehicle = 45.8 ± 3.7; dexamethasone = 64.7 ± 5.7* (pg/ml; n=8 per group; *P<0.01). We detected LTB_4 (~20 pg/ml) in only a few samples from dexamethasone-pretreated cultures.

3. Dexamethasone-up-regulated 5LOX expression requires the glucocorticoid receptor (GR)

We verified the expression of GRs in our preparation of CGNs with a specific anti-GR antibody. Using antisense technology, we significantly reduced the content of GRs in CGN cultures. GR antisense (AS) and the corresponding scramble (SCRM) oligonucleotides were injected directly into the culture medium twice at 12 h intervals. Twelve hours after the second treatment, cells were harvested for GR protein measurements. Application of antisense to GRs reduced the content of GR protein by more than 50% compared with SCRM-treated controls. Thus, we used antisense methodology to investigate whether alterations in GR content in CGN also alter the effects of dexamethasone on 5LOX expression (i.e., mRNA and protein levels). When GR AS or SCRM oligonucleotides were coadministrated with dexamethasone, GR AS but not SCRM suppressed the dexamethasone-induced increase in 5LOX mRNA and protein content (**Fig. 2**).

To further investigate whether the stimulatory effect of dexamethasone on 5LOX expression occurs via the

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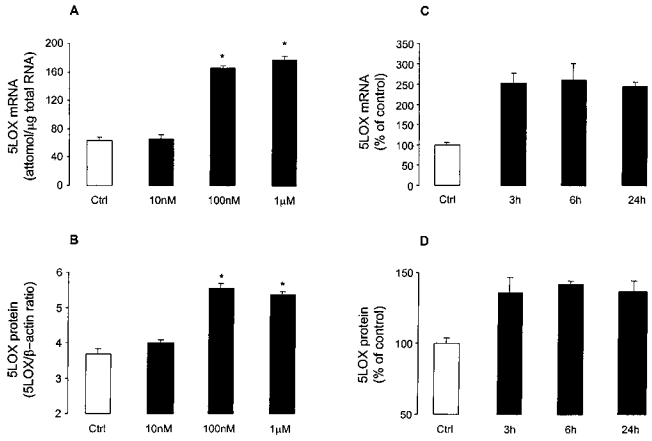

Figure 1. Effect of dexamethasone (Dex) on 5LOX mRNA and protein contents in primary cultures of rat cerebellar granule neurons (CGN). These cultures were treated with increasing concentrations of Dex for 24 h (*A, B*), or with 1 μM Dex for the periods indicated (*C, D*). Experiments were performed in 3–4 different culture preparations (*A, B*; mean±SE; **P*<0.01 vs. control; Ctrl) or in 2 different culture preparations (*C, D*; means expressed as % of the corresponding time controls: each time point had its own vehicle-treated group).

GRs, we used the GR antagonist RU486. After coadministration of 1 μM dexamethasone (or its vehicle) and 1 μM RU486 (or its vehicle) to CGNs for 24 h, 5LOX mRNA and protein levels were quantified. We found that RU486 significantly reduced dexamethasone-stimulated 5LOX expression. On the other hand, RU486 did not alter the basal levels of 5LOX mRNA and protein (Fig. 2).

4. Dexamethasone increases the half-life of 5LOX mRNA in CGNs

It has been suggested that the stimulatory action of dexamethasone on gene expression may include both increased transcription and post-transcriptional effects, such as prolongation of an mRNA's half-life. Thus, we examined 5LOX mRNA stability in CGN cultures treated for 24 h with dexamethasone or its vehicle and estimated the half-life of 5LOX mRNA after the inhibition of gene transcription with actinomycin D. Cultured neurons were treated with dexamethasone or its vehicle for 24 h before the addition of actinomycin D (10 μg/ml). Total RNA was isolated from vehicle- and dexamethasone-treated cells at different times after actinomycin D and 5LOX mRNA levels were measured by quantitative RT-PCR. The half-life ($t_{1/2}$) of the 5LOX mRNA in vehicle-treated cells was 4.0 ± 0.1 h, whereas in dexamethasone-treated CGNs the $t_{1/2}$ was 5.8 ± 0.2 h (i.e., 50% longer; n=3; P<0.01).

CONCLUSIONS AND SIGNIFICANCE

The results obtained in this study using primary cultures of rat cerebellar cells confirm our previous findings in the rat cerebellum in vivo: dexamethasone treatment increased the content of 5LOX mRNA and protein in cells of the mammalian central nervous system (CNS). However, we selected the in vitro system because it is better suited to mechanistic studies. Thus, the stimulatory effect of dexamethasone on 5LOX in CGNs was observed with concentrations as low as 100 nM, suggesting that 5LOX up-regulation occurs at physiologically relevant concentrations of glucocorticoid hormones.

Many of the effects of corticosteroids in the CNS are believed to be mediated via two types of corticosteroid receptors (mineralocorticoid receptors and GRs) to which dexamethasone binds. Previous studies with tritiated dexamethasone autoradiography and immunohistochemistry demonstrated nuclear localization of GRs in cerebellar granule cells in vivo. The functional involvement of GRs in the regulation of 5LOX expression in CGNs in vitro is indicated by our findings in experiments with GR antisense oligonucleotides and with the GR antagonist RU486; both were effective in inhibiting the up-regulation of 5LOX expression by dexamethasone. We performed these studies using two complementary methods because a single-method approach might generate inconclusive results, i.e., RU486 is a potent antagonist for both intracellular GRs and for progesterone receptors because it binds with high affinity to these receptors and causes transconformational differences in their ligand binding domains. Since GR antisense and RU486 both prevented dexamethasone from up-regulating 5LOX expression, it can be concluded that genomic mechanisms rather than a

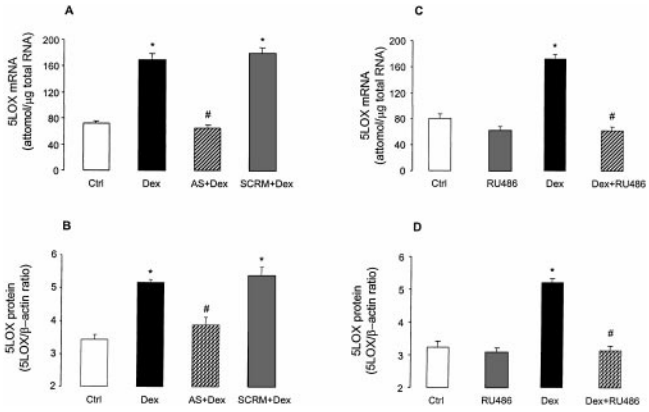

Figure 2. Glucocorticoid receptors are needed for stimulatory action of dexamethasone on 5LOX expression: antisense and pharmacological experiments. The GR antisense (AS: GGATTCTTTGGAGTCCAT) and the corresponding control scramble (SCRM: AATGCTCGCTTGATGTTG) oligonucleotides were dissolved in the culture medium and added to the cells twice (12 h intervals for 24 h; final concentration 10 μM); cells were processed 12 h after the second application of oligonucleotides; treatment with AS reduced the content of GR-immunoreactive protein. Coadministration of dexamethasone (Dex; 1 μM) with oligonucleotides was performed in panels *A* and *B* and with 1 μM RU486 (GR antagonist) in panels *C* and *D*. Both AS and RU486 prevented the stimulatory action of Dex; SCRM was ineffective (n=3 different preparations; mean±SE; P<0.05: *vs. Ctrl, #vs. Dex).

nongenomic action that involves the activation of putative membrane receptors for glucocorticoids mediated the effects of dexamethasone we observed in CGNs. However, it should be stressed that no obvious glucocorticoid response element has been found in the sequence of the 5LOX gene and its promoter. Thus, it remains to be established whether such a response element is located elsewhere in the genome and in relation to the 5LOX gene.

Using various in vitro models of cell cultures, others have also observed 5LOX up-regulation in response to dexamethasone treatment. For example, 5LOX mRNA and protein were both increased after dexamethasone treatment in human mast cells and in human monocytes and THP-1 cells. In those cell cultures, dexamethasone also increased the capacity of these cells to produce LTs, and the authors concluded that this synthetic glucocorticoid increased 5LOX gene expression. Our primary cultures of CGNs express 5LOX and the 5LOX-activating protein FLAP. Nevertheless, we found that they do not produce detectable levels of LTs unless provided with exogenous arachidonic acid. When provided with arachidonic acid and in the presence of the calcium ionophore, these cells produced cysLTs, and their capacity to synthesize cysLTs was increased by dexamethasone pretreatment. Thus, we also conclude that collectively our data on 5LOX mRNA, 5LOX protein, and LT synthesis are consistent with the proposed stimulatory action of dexamethasone on 5LOX gene expression (**Fig. 3**).

Our finding that dexamethasone pretreatment prolonged the half-life of 5LOX mRNA suggests it is possible that mechanisms that regulate RNA stability could also contribute to the increase in 5LOX gene expression we observed. Such mechanisms are also likely to include a direct action of dexamethasone on 5LOX mRNA or on mRNA-selective proteins/enzymes. Glucocorticoids have already been shown capable of enhancing the stability of other mRNAs, such as that of insulin receptors and growth hormone. Even though

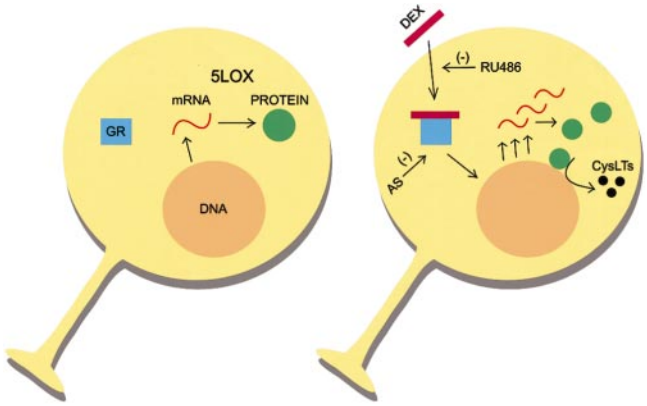

Figure 3. Schematic presentation of main findings. At resting, CGNs express glucocorticoid receptors (GR) as well as 5LOX mRNA and protein. Exposure of CGN to dexamethasone (DEX) increases 5LOX mRNA and protein expression and results in the increased capacity of these cells to synthesize cysLTs. The stimulatory action of DEX can be inhibited (−) either by RU486 (GR antagonist) or by decreasing the cell content of GR with the GR-specific antisense oligonucleotide (AS).

the molecular mechanisms of the hormonal regulation of RNA stability are not very well understood, it has been speculated that glucocorticoids may induce a factor that associates with an RNA element in the 3′untranslated region (3′UTR) of these genes.

The functional significance of our findings for the physiology and/or pathology of the CNS remains to be fully characterized. However, available data point to possible roles for the 5LOX pathway in brain aging, neurodegeneration, seizures, synaptic activity, neurogenesis, and neurodevelopment. It is also important to elucidate whether enzymatic or even nonenzymatic actions of 5LOX protein are relevant for CNS functioning. FJ

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