Glucocorticoid hormones downregulate histidine decarboxylase mRNA and enzyme activity in rat lung

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Zahnow, Cynthia A., Pertti Panula, Atsushi Yamatodani, and David E. Millhorn. Glucocorticoid hormones downregulate histidine decarboxylase mRNA and enzyme activity in rat lung. Am. J. Physiol. 275 (Lung Cell. Mol. Physiol. 19): L407–L413, 1998.—Histidine decarboxylase (HDC) is the primary enzyme regulating histamine biosynthesis. Histamine contributes to the pathogenesis of chronic inflammatory disorders such as asthma. Because glucocorticoids are effective in the treatment of asthma, we examined the effects of 6 h of exogenously administered dexamethasone (0.5–3,000 µg/kg ip), corticosterone (0.2–200 mg/kg ip), or endogenously elevated corticosterone (via exposure of rats to 10% oxygen) on HDC expression in the rat lung. HDC transcripts were decreased ~73% with dexamethasone treatment, 57% with corticosterone treatment, and 50% with exposure to 10% oxygen. Likewise, HDC enzyme activity was decreased 80% by treatment with dexamethasone and corticosterone and 60% by exposure to 10% oxygen. Adrenalectomy prevented the decreases in HDC mRNA and enzyme activity observed in rats exposed to 10% oxygen, suggesting that the adrenal gland is necessary for the mediation of hypoxic effects on HDC gene expression. These results demonstrate that corticosteroids initiate a process that leads to the decrease of HDC mRNA levels and enzyme activity in rat lung.

Histamine is produced by the decarboxylation of L-histidine via the pyridoxal-requiring enzyme histidine decarboxylase (HDC). In eukaryotes, HDC is the primary enzyme responsible for synthesizing histamine and is consequently a crucial regulatory step for histamine biosynthesis. Histamine contributes to the pathogenesis of obstructive lung diseases such as asthma primarily through its actions on the inflammatory response, bronchial and vascular smooth muscle, capillary and airway epithelial permeability, and the secretion of mucus and serous fluid. Alveolar hypoventilation leading to arterial hypoxemia and increased serum levels of glucocorticoids are a common consequence of airway constriction associated with asthma. Glucocorticoids are among the most effective anti-inflammatory agents available for the treatment of asthma. These steroids reduce the symptoms of asthma by 1) decreasing airway secretions via actions on mucous glands and vascular permeability, 2) decreasing airway inflammation through inhibitions of inflammatory cell recruitment into the airways and vasoconstriction of the vasculature, 3) decreasing airway reactivity, and 4) improving airway integrity and thus function.

The anti-inflammatory actions of glucocorticoids are mediated through glucocorticoid receptors (GRs), which are primarily localized to the cytoplasm of target cells, but, upon binding of steroid hormone, the activated complex moves to the nucleus where it dimerizes and binds to the promoter region of DNA at consensus sites termed glucocorticoid response elements (GREs) and either increases or decreases transcription rates. Among the genes relevant to asthma, glucocorticoids increase the transcription of lipocortin-1, β2-adrenoreceptor, endothelin-1, natural killer cell receptors, and adhesion molecules (intercellular adhesion molecule-1; see Ref. 2). Despite extensive accumulated knowledge about the anti-inflammatory actions of corticosteroids, relatively little is known about the molecular mechanisms by which these steroid hormones mediate their effects on the inflammatory mediator histamine. We propose that glucocorticoids, endogenously elevated during hypoxia associated with respiratory illness, downregulate HDC gene expression. The resulting changes in availability or storage of histamine may then contribute to the attenuation of the inflammatory response. To address this question in rat lung, we have studied the regulation of HDC mRNA, enzyme activity, and histamine levels by glucocorticoids that were either exogenously administered or endogenously elevated by exposure to 10% oxygen.

MATERIALS AND METHODS

Animals. Sprague-Dawley male rats were housed in a controlled environment of 22°C and subjected to a 12:12-h dark-light cycle. They were fed pelleted Agway rat chow and were given water ad libitum. At 30 days of age, the rats were anesthetized via an intramuscular injection of a 5:1 mixture of ketamine hydrochloride (Ketalar; 100 mg/ml)-xylazine (Rompun; 20 mg/ml) and bilaterally adrenalectomized or sham operated. The rats were postoperatively maintained with 0.85% saline for 2 wk. At 44 days of age, the adrenalectomized, nonadrenalectomized (sham-operated), and control (no surgery) rats were subjected to the various experimental procedures and then killed by decapitation. To minimize any responses that might have been caused by circadian changes in glucocorticoid levels, rats were killed between the hours of
1600 and 1800. Trunk blood and tissues were collected and frozen at −80°C for later processing.

Steroid treatment. For the dose-response studies, adrenalectomized, 44-day-old male rats were intraperitoneally injected with a single bolus dose of either 0.5–3,000 µg/kg dexamethasone (Steraloids) or 0.2–200 mg/kg corticosterone (Sigma). The steroid was first dissolved in 100% ethanol (EtOH) and diluted to the appropriate dose using 1× PBS. The final concentration of the vehicle was either 10% EtOH-90% PBS or 20% EtOH-80% PBS. Nonadrenalectomized (sham-operated) rats were treated with vehicle alone. Six hours after treatment, rats were killed, and tissues were removed and stored at −80°C for later processing of HDC mRNA, HDC enzyme activity, and histamine concentrations.

Hypoxia exposure. Forty-four-day-old male rats were placed in a sealed Plexiglas chamber and exposed continuously to humidified 10% oxygen for 6 h. Rats exposed to 6 h of 10% oxygen in a sealed Plexiglas chamber and exposed continuously to 10% oxygen levels were monitored within the chamber with an oxygen sensor (Becton Dickinson Medical Systems). Rats were provided with food and water ad libitum. Control (normoxic) rats were kept in an identical chamber, and all rats were subjected to a 12:12-h dark-light cycle. At the end of the exposure to normoxia or hypoxia, rats were removed and killed by decapitation.

Isolation of polyadenylated mRNA and Northern blot analysis. Poly(A)−enriched RNA was isolated and purified from lung and brain tissue by a modification of the methods of Badley et al. (1) and Patel and Kurkinen (17). Tissue was homogenized in a lysis buffer containing protease K (Boehringer Mannheim), and mRNA was purified by affinity chromatography using oligo(dT) cellulose (type 2; Collaborative Research). mRNA (10–20 µg) was resuspended in 10 µl of a glyoxal mixture (75 µl of DMSO, 30 µl of glyoxal, 15 µl of 0.2 M Na2HPO4, and 30 µl of H2O) and heated to 50°C for 1 h. Two and one-half microliters of 10× dye mix (50% sucrose, 0.25% bromphenol blue, 1/10 vol, and 1× Tris-borate-EDTA) were added to the samples, and the RNA was size fractionated via electrophoresis in a 1% agarose gel prepared in 10 mM Na2HPO4 buffer. The RNA was then transferred in 20× sodium chloride-sodium citrate buffer (SSC) to a Biotrans nylon membrane. The membrane was prehybridized at 42°C for 2 h in a solution containing 50% formamide, 4.8× SSC, 4.8× Denhardt’s solution, 48 mM Na2HPO4, 1% glycine, 0.5% SDS, and 0.24 mg/ml salmon sperm DNA. Hybridization proceeded at 42°C overnight in a solution containing 50% formamide, 4.8× SSC, 1× Denhardt’s solution, 20 mM Na2HPO4, 0.1 mg/ml salmon sperm DNA, 0.5% SDS, 10% dextran sulfate, and 2 × 106 counts/min of radioactively labeled probe per milliliter of hybridization solution. Rat HDC cDNA was labeled using [γ-32P]ATP (3,000 Ci/mmol; Amersham) by nick translation (GIBCO BRL) as recommended by the supplier. β-Actin oligonucleotide was end labeled using T4 polynucleotide kinase (Promega) and [γ-32P]ATP (New England Nuclear).

To ensure that equivalent amounts of RNA were electrophoresed and transferred, the level of β-actin mRNA in each lane was measured. Densitometric measurements were made from the autoradiograms of the HDC and β-actin hybridized Northern blots. Each measurement of HDC mRNA was normalized to measurement of β-actin in the same sample and was then expressed as a percentage of control or control mean (±SE). Differences between groups were analyzed using the nonparametric, two-tailed Mann-Whitney statistical test.

Extraction and HPLC measurement of histamine. Frozen tissues were sonicated in 10 volumes of 2% perchloric acid for 15–20 s, at maximal setting, using a sonifier cell disruptor (Heat Systems; Ultrasonics) and were centrifuged at 4°C for 30 min at 12,000 rpm in an Eppendorf microcentrifuge. The histamine content of the resultant supernatant was measured as previously described using an HPLC fluorometric method (25).

Extraction of HDC and assay for enzyme activity. Frozen tissues were sonicated at maximum setting in 8–10 volumes of an ice-cold solution (100 mM potassium phosphate buffer, pH 6.8, 0.1 mM dithiothreitol, 0.01 mM pyridoxal 5’-phosphate, 1% polyethylene glycol-300, 20 µg/ml phenylmethylsulfonyl fluoride, and 0.01 mM aminoguanidine) until completely disrupted (23, 24) and centrifuged at 4°C for 30 min at 12,000 rpm in an Eppendorf microcentrifuge. The supernatant was transferred into dialysis tubing (Spectra/Por MWCO 12–14,000) and dialyzed three times against 100 volumes of the sonication solution at 4°C for 20 h. Each extract was then divided into two 100-µl portions for experimental and blank samples as well as a 10-µl aliquot for Bradford protein assay (Bio-Rad). γ-Globulin served as the protein standard for generation of the standard curve for the Bradford assay. One hundred microliters of l-histidine (1 mM) were added to serve as substrate for the enzyme activity assay, and 100 µl of water were added to the blank samples. The reaction proceeded at 37°C for 4 h and was stopped by the addition of 20 µl of 20% perchloric acid. Samples were centrifuged at 12,000 rpm for 15 min, and the histamine levels were measured by HPLC (25).

Corticosterone radiomunoassay. Trunk blood was collected from experimental rats into 50-ml polystyrene tubes, allowed to clot on ice, and centrifuged to separate the serum. Serum was removed and stored at −80°C until assayed. Total serum corticosterone was measured using an Immucor double antibody (129) radiomunoassay kit from ICN Biomedicals.

RESULTS

In rat lung, three transcripts of sizes 3.5, 2.6, and 1.6 kb were detected using rat HDC cDNA as the probe (Fig. 1, A and B). Changes occurring in the levels of the 3.5- and 2.6-kb transcripts were considered indicative of changes in HDC mRNA, whereas the 1.6-kb transcript served as an internal control since it remained unchanged throughout the experiments. The levels of these two larger transcripts were decreased in a dose-dependent manner in response to a 6-h treatment of adrenalectomized rats with a bolus dose of dexamethasone (0.5–3,000 µg/kg ip; Fig. 1, A and C). These transcripts were significantly decreased to below control values at 5.0 µg/kg dexamethasone (P < 0.05), with a maximum decrease of 73% observed at 2,000 µg/kg dexamethasone (P < 0.05; Fig. 1C). Similarly, HDC enzyme activity was decreased in a dose-dependent manner and was maximally reduced to 80% below that of the vehicle control value at 2,000 µg/kg dexamethasone (P < 0.01; Fig. 2A). Unfortunately, changes in HDC protein levels could not be determined due to the unavailability of an antibody to HDC. No dose of dexamethasone tested in these rats altered endogenous lung histamine levels when compared with the vehicle control level (Fig. 2B).

All experiments in this study were conducted for 6 h because it was determined that the maximal decrease in both HDC mRNA and enzyme activity occurred after 6 h of treatment with a single bolus dose of dexametha-
Northern blot of HDC poly(A)+ RNA purified from the lungs of adrenalectomized rats injected with a single bolus dose of dexamethasone (µg/kg ip) and killed 6 h later. Both vehicle (adrenalectomized) and sham (nonadrenalectomized) rats were injected with vehicle only (20% ethanol (EtOH)-80% PBS ip). Three HDC hybridizing transcripts of sizes 3.5, 2.6, and 1.6 kb were detected using rat HDC cDNA as probe. 

To address the question of whether HDC gene expression is regulated by both the synthetic glucocorticoid dexamethasone and the endogenous glucocorticoid corticosterone, adrenalectomized rats were treated with a single bolus dose of corticosterone (0.2–200 mg/kg ip). Radioimmunoassay analysis of the serum from these rats demonstrated that the administered corticosterone produced serum levels that fall within the normal physiological range for rat corticosterone levels (vehicle, 5.3 ± 1.6 ng/ml; 0.2 mg, 10.5 ± 5.4 ng/ml; 2 mg, 7.75 ± 5 ng/ml; and 20 mg, 124 ± 94 ng/ml), and treatment with the highest dose of corticosterone (200 mg/kg ip) resulted in a pharmacological serum corticosterone concentration of ~1,839 ± 258 ng/ml. As was observed with dexamethasone, corticosterone (0.2–200 mg/kg ip) caused a dose-dependent decrease in both the 3.5- and 2.6-kb HDC transcripts, with a maximal decrease of 57% below that of the vehicle control value (P < 0.01) observed at the 200 mg/kg dose (Fig. 4). Additionally, 20 and 200 mg/kg of corticosterone decreased HDC enzyme activity by ~80% (P < 0.002), whereas histamine levels remained unchanged compared with vehicle control (Fig. 5, A and B). Thus similar changes in HDC gene expression were obtained with exogenously administered dexamethasone and corticosterone.

The administration of high levels of dexamethasone and corticosterone might reflect pharmacological rather than physiological serum levels of the steroid in question. Therefore, in an effort to study the regulation of HDC in a situation where glucocorticoid levels are elevated, but more closely approximate physiological concentrations, nonadrenalectomized rats were subjected to hypoxia, a general physiological stress. Continuous exposure to 10% oxygen for 1 h generated elevated levels of serum corticosterone of ~400 ng/ml, which were four times that of the serum corticosteroid level for normoxic rats. After 6 h of exposure, the serum steroid concentrations were reduced to ~200 ng/ml, probably due to negative feedback by the elevated corticosterone.

The rat’s response to hypoxic exposure and the resultant changes in the regulation of HDC gene expression in lung are complex. In an effort to eliminate and thereby identify some of the hormonal factors that may be involved in this response, the adrenal glands, the primary source of corticosterone in the rat, were removed, and the rats were then continuously exposed for 6 h to 10% oxygen. Both mRNA and enzyme activity were decreased ~60 and 53% (P < 0.005), respectively, in the lungs of nonadrenalectomized, intact (sham) rats exposed to hypoxia for 6 h; however, adrenalectomized rats did not downregulate HDC mRNA or enzyme activity (Figs. 6 and 7A). These data indicate that the adrenal gland is necessary for the mediation of hypoxic effects on HDC gene expression. Histamine levels again remained unchanged in adrenalectomized and nonadrenalectomized rats compared with the normoxic control value (Fig. 7B).

**DISCUSSION**

Multiple transcripts have previously been reported for HDC. HDC transcripts of 3.5, 2.6, and 1.6 kb have been observed in rat brain (3) and fetal rat liver (10); however, the latter does not contain the 1.6-kb transcript. Similarly, HDC transcripts of 3.7, 3.2, 2.9, and 1.6 kb have been detected in Drosophila head (5). Additionally, an HDC transcript of ~2.4 kb has been...
cloned and sequenced from a human basophilic leukemia (KU-812-F) cell line (26) and a human erythroleukemia cell line (28). There are very few published studies that have addressed the basis for this transcript heterogeneity. One such study investigated the functional analysis of two HDC cDNA clones representing the 3.4- and 2.4-kb HDC transcripts of a human basophilic cell line (KU-812-F). It was determined that the 3.4-kb mRNA is an alternately spliced transcript in which the 7th intron (824 bp) is not spliced out and contains an in-frame translation stop codon resulting in a truncated protein, whereas the 2.4-kb mRNA was found to encode a functional HDC enzyme (14, 27). Likewise, it was demonstrated in COS cells that the 2.6-kb HDC mRNA, isolated from rat fetal liver, encodes a functional HDC (10). No published functional data exist for the smallest HDC transcript (1.6 kb). This mRNA could code for a protein highly related to HDC and may therefore cross-react with the HDC cDNA. Analysis of the 1.6-kb transcript using oligonucleotides directed toward internal regions as well as the 5' and 3'-ends has led us to speculate that the 1.6-kb transcript may be different from the published HDC cDNA sequences (data not shown). Consequently, during our analysis of HDC mRNA, we have focused on the changes occurring in the levels of the 3.5- and 2.6-kb transcripts and have used the 1.6-kb transcript as an internal control because its levels have remained unchanged throughout our experiments.

Our results from this study demonstrate that corticosteroids initiate a process that leads to the decrease in HDC mRNA levels and enzyme activity in rat lung. Steady-state levels of mRNA usually reflect a balance between the rate of RNA transcription and RNA stability. We have not determined which of these mechanisms might account for the decrease in HDC mRNA, but we suspect that a reduction in transcription rate may be involved. Sequence analysis of the rat HDC gene by our laboratory has identified several putative regulatory elements, including activator protein (AP)-1, AP-2, Oct 1, Sp1, CCAAT boxes, hypoxia inducible factor (HIF)-1 binding sites, and five GRE-like consensus sites (unpublished observations). There are several molecular mechanisms that could account for a decrease in transcription rate. It is well established that the GR interacts with c-Jun homodimers or c-Jun-c-Fos heterodimers (AP-1) before DNA binding and results in an inhibition of both of their abilities to activate transcription (15). Additionally, the nuclear ratio of Jun to Fos is important for determining whether interactions with the GR will have a negative or positive effect on transcription. Another possible mechanism is that GREs on the HDC gene function as a composite response element in which the GR not only binds to...
DNA but also interacts with other trans-acting factors to inhibit transcriptional activity. Jun/Fos and HIF are likely candidates because AP-1-like binding sites and HIF-1 binding sites have been tentatively identified near several GRE-like sequences within the HDC gene (unpublished observations).

Regulation of HDC gene expression by glucocorticoids may be occurring via direct or multiple indirect pathways. It is well established that GRs are present in rat lung (4). It is possible that the GR may activate a different set of genes or a second messenger pathway that in turn regulates HDC and can be additionally activated by something other than GR.

In experimentation with intact animals, it is difficult to isolate the variable under investigation from other physiological factors that may also affect the condition being evaluated. Our data support the hypothesis that glucocorticoids, in part, mediate the hypoxic downregulation of HDC mRNA because the regulatory response is blocked in adrenalectomized animals. However, we cannot discount the possibility that factors other than glucocorticoids may be influencing the regulation of HDC gene expression. For example, hypoxic stress is associated with elevated serum levels of corticosterone, ACTH, and catecholamines (18, 21). ACTH levels were not measured in our study but are reported to be elevated during hypoxia (18, 21) and downregulated (via negative feedback) as doses of exogenously administered steroids are increased. If so, it seems unlikely that these opposite fluctuations in the levels of ACTH could account for the consistent downregulation of HDC mRNA and enzyme activity during both experimental paradigms. The adrenal gland is the sole source
of glucocorticoids and mineralocorticoids and also produces androgens, estrogens, progestins, and catecholamines. This gland is necessary for mediation of the regulatory effects of hypoxia because adrenalectomized rats lose the ability to downregulate HDC gene expression in response to hypoxic exposure (Figs. 6 and 7, A and B). Studies have demonstrated that removal of the adrenal gland can exacerbate the inflammatory response, suggesting that endogenous steroids can suppress inflammation (22). It is possible, however, that other adrenal steroids or catecholamines might be involved in the regulation of HDC gene expression.

Interestingly, endogenous histamine concentrations within the rat lung did not change significantly during any of the experimental conditions used in this study. Several explanations may account for this observation. In the lung, the majority of histamine is stored within mast cell granules and exhibits a very slow turnover rate (13). The 80% decrease observed in HDC enzyme activity may not produce an equal decrease in histamine stores over a 6-h period. In order for the amine to be metabolized, it must be released from its storage granules. Degranulation of mast cells is a complex process, and although hypoxia has been shown to induce histamine release (7), it is not known whether the experimental paradigms used in this study caused degranulation. Corticosteroids do not directly inhibit histamine release from lung mast cells; however, steroid treatment is associated with a decrease in mucosal mast cell numbers (2). If a decrease in mast cell number was responsible for the observed decreases in HDC mRNA and enzyme activity, a decrease in histamine levels might also be expected to occur. Additionally, the activity of histamine-metabolizing enzymes may also be downregulated by glucocorticoids. We speculate that, over longer periods of time, the reduced levels of HDC mRNA and enzyme activity will result in a decreased production and consequently a decreased tissue level of histamine.

In agreement with our results, several studies in rat and mouse lung have shown that glucocorticoid hormones may be involved in the reduction of HDC enzyme activity (8, 19, 20). In contrast to our studies in rat lung, glucocorticoids have been shown to activate HDC gene expression in mastocytoma and basophilic leukemia cell lines. Experiments with cell lines provide valuable information but must be interpreted with caution because they do not precisely duplicate the conditions and multiple variables that exist in the whole animal.

Dexamethasone increased both histamine content and the de novo synthesis of HDC from cultured mouse mastocytoma P815 cells and cultures of fetal rat hepatic cells (9, 16). This regulation was further studied by examining the synergistic effects of glucocorticoids and the protein kinase C activator 12-O-tetradecanoylphorbol 13-acetate (TPA) on HDC synthesis from mouse P815 cells (11). Previously, it had been shown that activation of protein kinase C by phorbol 12-myristate 13-acetate or oleoyl acetylglyceryl led to increased synthesis of HDC in rat basophilic leukemia cells, RBL-2H3 cells, and human basophilic leukemia cells, KU-812-F (12, 14). In accordance, the TPA studies demonstrated that protein kinase C may be involved in the glucocorticoid-induced synthesis of HDC in mouse mastocytoma cells (11). Taken together, these results suggest that the effects of glucocorticoid hormones on HDC gene expression may be tissue or cell specific.

In summary, glucocorticoid hormones do not appear to be responsible for maintaining the constitutive expression of HDC in rat lung but do appear to be involved in either the inactivation of HDC gene transcription or reduction in HDC transcript stability. Additionally, glucocorticoids reduce HDC enzyme activity, and it is not known whether this reduction is due to a decrease in protein levels or the posttranslational inactivation of enzyme activity. This downregulation of HDC gene expression might, in part, account for the effectiveness of glucocorticoids in the treatment of inflammatory diseases such as asthma.

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