Blocking the Mineralocorticoid Receptor Improves Effectiveness of Steroid Treatment for Low Back Pain in Rats

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ABSTRACT

Background: Localized inflammation of lumbar dorsal root ganglia (DRG) may contribute to low back pain. Local injections of corticosteroids used for low back pain are sometimes ineffective. Many corticosteroids activate not only the target glucocorticoid receptor (GR) but also the mineralocorticoid receptor (MR), which may have proinflammatory effects countering the effects of GR activation.

Methods: A low back pain model was implemented in rats (n = 6 to 10 per group) by locally inflaming the L5 DRG. Sensory neuron excitability and mechanical hypersensitivity of the hind paws were measured. Tested steroids were applied locally to the inflamed DRG or orally.

Results: The selective MR blocker eplerenone reduced pain behaviors when given orally starting at the time of surgery, or starting 7 days later. The highly GR-selective agonist fluticasone, applied locally to the inflamed DRG, was much more effective in reducing mechanical hypersensitivity. The MR/GR agonist 6-α methylprednisolone, commonly injected for low back pain, reduced mechanical hypersensitivity when applied locally to the DRG but was less effective than fluticasone. Its effectiveness was improved by combining it with local eplerenone. All tested steroids reduced hyperexcitability of myelinated sensory neurons (n = 71 to 220 cells per group) after inflammation, particularly abnormal spontaneous activity.

Conclusions: This preclinical study indicates the MR may play an important role in low back pain involving inflammation. Some MR effects may occur at the level of the sensory neuron. It may be useful to consider the action of clinically used steroids at the MR as well as at the GR. (Anesthesiology 2014; 121:632-43)

LOW back pain is common and difficult to treat.1,2 Although the etiology is often unknown, inflammation of the lumbar dorsal root ganglia (DRG), for example, secondary to an immune response to the nucleus pulposus, may contribute to some forms of low back pain.3,4 Sensory neurons and their surrounding glia synthesize, release, and respond to cytokines and other molecules originally described as components of the immune system inflammatory response.5 Several preclinical models of low back pain involve inducing local inflammation of the lumbar DRG.6

A common treatment for some forms of low back pain involves local injections of corticosteroids. Randomized clinical trials of such treatments have had mixed results; often steroid injections are effective only in the short term.7,8 The nominal target of corticosteroid drugs is the glucocorticoid receptor (GR). However, recent in vitro studies show that many clinically used steroids (e.g., 6-α methylprednisolone and triamcinolone) can also activate the mineralocorticoid receptor (MR) with similar potency.9,10 The MR was originally viewed only as the target of aldosterone, promoting sodium reabsorption in the kidney. However, this receptor has been detected in other cell types including cardiomyocytes,11 brain neurons,12 and DRG neurons.13 In many tissues, the MR may be proinflammatory.14,15 Some proinflammatory effects may be due to receptors in macrophages, where MR activation promotes production of proinflammatory cytokines and tissue destruction (type I inflammation), whereas GR activation promotes tissue remodeling and wound repair (type II inflammation).16 In some nonrenal tissues, the MR may be...
activated primarily by the endogenous corticosteroids corticosterone (rodents) or cortisol (humans) rather than aldosterone because these tissues lack the glucocorticoid-inactivating enzyme 11β-hydroxysteroid dehydrogenase type 2 that, in the kidney, ensures that only aldosterone activates the MR.16

In this study, we used our rat model of low back pain induced by local inflammation of the DRG (LID) by depositing a drop of the immune activator zymosan over a single lumbar DRG. This causes prolonged mechanical hypersensitivity, rapid up-regulation of proinflammatory cytokines, and hyperexcitability of small-diameter sensory neurons.17 We showed that the MR in sensory neurons is activated in this model, and that applying a MR antagonist locally to the inflamed DRG reduced the pain behaviors and hyperexcitability.13 The MR antagonist eplerenone used has much better selectivity for MR over GR compared with previous agents18 and is currently approved for use in hypertension and heart failure.

In the current study, we examined the role of the MR and GR in our preclinical low back pain model by examining the effect of several clinically used steroids. We first determined whether a more clinically relevant oral route of eplerenone administration would also be effective in our low back pain model. We also tested the hypothesis that local DRG application of highly GR-selective drugs would be more effective in reducing pain behaviors than drugs that activate both MR and GR in vitro, and that the effectiveness of the latter could be improved by combining them with an MR antagonist.

Materials and Methods

Animals
All surgical procedures and the experimental protocol were approved by the Institutional Animal Care and Use Committee (Cincinnati, Ohio) and adhered to the guidelines of the Guide for the Care and Use of Laboratory Animals. Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) were used for all experiments. Rats were housed one or two per cage at 22° ± 0.5°C under a controlled diurnal cycle of 14-h light and 10-h dark with free access to water and food. Male rats weighing 150 to 200 g at the start of the experiment were used for behavioral experiments. Female rats weighing 100 to 150 g were used for electrophysiological experiments. We have previously shown that behavioral sensitivity in response to DRG inflammation does not differ between these two groups at the time points used for electrophysiological recording.19 Behavioral testing was also conducted in younger female animals for 6-α methylprednisolone and is presented in Supplemental Digital Content 1, http://links.lww.com/ALN/B50. This steroid was chosen for more extensive testing in females because it activates both MR and GR in vitro.

Behavioral Testing
Animals were tested every other day for two trials before implementing the LID model (baseline) and after surgery as indicated. The average of the baseline values is plotted as postoperative day (POD) 0.

Mechanical sensitivity of the hind paw was tested with von Frey filaments using the up-and-down method.20 A cutoff value of 15 g was used. This size rarely evokes a response in normal animals; higher filament values were not tested as they sometimes caused lifting of the paw. Cold sensitivity was scored as the percentage (of six trials) of responses to a drop of acetone applied to the ventral surface of the hind paw. The response to light brush strokes was scored as percentage of brisk withdrawals (of six trials) to a normally innocuous mechanical stimulus that fails to evoke responses in normal animals (a wisp of cotton stroked across the plantar surface of the hind paw). When observed, such responses to acetone or light brush strokes consisted of several rapid flicks of the paw and/or licking and shaking of the paw; walking movements were not scored as positive responses. Behavior data for each individual drug were compared with that from non-drug control animals from the same experiment, measured side-by-side. The experimenter was not blinded with respect to the treatment. Animals were housed two per cage; one received the LID surgery, whereas the second received LID surgery plus indicated steroid treatment. These assignments were done at random before baseline testing.

Surgical Procedures for Localized Inflammation of DRG (LID)
The surgery was performed as previously described.21 The L5 DRG was inflamed by depositing the immune activator zymosan (2 mg/ml, 10 μl in incomplete Freund’s adjuvant) over the L5 DRG via a small needle inserted into the L5 intervertebral foramen. For animals used for electrophysiological experiments, the L4 DRG was also inflamed. For LID with systemic oral eplerenone, pharmaceutical grade eplerenone was given by gavage, 100 mg kg−1 day−1. The dose was chosen based on previous experiments in rats.22–24 The vehicle was 0.5% carboxymethylcellulose (catalog C-5013; Sigma, St. Louis, MO); 0.1% Polysorbate 80 (Tween 80; Sigma) in distilled water. Because steroids are very insoluble in water, they were applied locally to the inflamed DRG by adding them to the 10 μl of oily incomplete Freund’s adjuvant plus zymosan used to inflame the DRG. The method was based on published studies,25,26 in which steroid micro-pellets implanted into the brain had a diffusion radius of approximately 750 μm and duration of action of 5 to 7 days. We adjusted the amounts of agonists used based on EC50 values at the GR for the individual steroids used taken from published in vitro studies. Amounts used were 30 μg fluticasone (Tocris, Bristol, United Kingdom)27–29 and 30 μg 6-α methylprednisolone (catalog M0639; Sigma).10 For the MR antagonist eplerenone (Tocris), 500 μg was used,13 reflecting its lower in vitro potency for inhibition of the MR.

Electrophysiological Recording in Isolated Whole DRG Preparation
Spontaneous activity, action potential parameters, and input resistance were measured in current clamp mode at 36° to
37°C using microelectrodes in an acutely isolated whole DRG preparation, as previously described.21 This preparation allows neurons to be recorded without enzymatic dissociation, with the surrounding satellite glia cells and neighboring neurons intact.30,31 The DRG was continuously perfused with artificial cerebrospinal fluid containing the following: NaCl, 130 mM; KCl, 3.5 mM; NaH2PO4, 1.25 mM; NaHCO3, 24 mM; dextrose, 10 mM; MgCl2, 1.2 mM; CaCl2, 1.2 mM; pH = 7.3, bubbled with 95% O2–5% CO2. DRGs were perfused for 15 to 30 min after being removed from the animal, before recording began. Any spontaneous activity observed after impalement of the cell was recorded first and reconfirmed at the end of the recording period. An example of spontaneous activity is shown in figure 1. Action potential threshold, and width were measured during the response to the smallest current that evoked an action potential (rheobase) during a 75-ms depolarization. The action potential voltage threshold was defined as the first point on the rising phase of the spike at which the rate of change in voltage exceeded 1/10th of the maximum value. An example is shown in figure 1. Rheobase for spontaneously active cells was defined as zero. Data are from cells with action potential duration less than 1.5 ms, which in a previous study,19 we found consisted of 99% myelinated cells (based on dorsal root conduction velocity) both before and 3 days after DRG inflammation. Few effects were observed in cells with action potential duration more than 1.5 ms, a criterion that captured 97 to 98% of all C-cells both before and after DRG inflammation.

**Data Analysis**

Statistical analyses were performed using GraphPad Prism software (GraphPad Prism, La Jolla, CA). Nonparametric methods were used for data that did not show a normal distribution (D’Agostino and Pearson omnibus normality test). For electrophysiological data, comparison of the three experimental groups in each experiment (cells from normal, inflamed, and inflamed plus steroid-treated DRGs) was done using ANOVA (parametric) or Kruskal–Wallis test (nonparametric). Bonferroni (parametric) or Dunn post-test (nonparametric) was then used to compare the data from inflamed DRGs with that from the other two groups. Two-tailed hypothesis testing was used. Significance of percentage of cells with spontaneous activity was determined using the chi-square test. On the basis of previous experience, cells for all electrophysiological data were obtained from at least three different animals per group to avoid problems with interanimal variability and to obtain enough C-cells for analysis. The number of animals and cells used for each group in the electrophysiological data is indicated in the figure legends. For behavioral time course data, a two-way repeated-measures ANOVA with Bonferroni post-test was used to determine whether groups differed and if so on which days. On the basis of our previous experience on the variability of the behavior measures used, all behavioral data were obtained using at least six animals per group. There were no missing data points in the behavioral experiments. In a small number of cells (5.2% overall), the threshold measurement was not used due to incomplete bridge balance correction. Significance was ascribed for P value less than 0.05. Levels of significance are indicated by the number of symbols, for example, *P = 0.01 to <0.05; **P = 0.001 to 0.01; ***P < 0.001. Data are presented as mean ± SEM.

**Results**

**Oral Eplerenone Starting from POD7 Reduced Pain Behaviors Induced by Local DRG Inflammation**

Our previous study showed that locally applying eplerenone to the inflamed DRG reduced the pain behavior.13 Currently, eplerenone is available only as an oral formulation. In this
study, we investigated a more clinically relevant method of drug delivery by giving eplerenone systemically by oral gavage. First, we examined the effect of oral eplerenone, given daily starting from POD7, on mechanical hypersensitivity induced by local inflammation of the L5 DRG. Xie et al.\textsuperscript{19} showed that DRG inflammation leads to ipsilateral mechanical hypersensitivity starting as early as POD1 and continuing for more than 30 days. When we administered oral eplerenone starting from POD7, when hypersensitivity was already well established, the mechanical hypersensitivity was reduced on POD 8 and POD 14 ($P < 0.01$; fig. 2A). Abnormal withdrawal responses to light stroke stimuli were also blocked on POD 8 and POD10 ($P < 0.001$; fig. 2B). Cold hypersensitivity as measured by the acetone test was significantly less on POD 8 and POD10 (fig. 2C) in the drug-treated group; however, this finding was inconclusive because, in this cohort of rats, the responses to this test were less reproducible from group to group and some differences between the two groups were also observed before drug treatment.

**Oral Eplerenone Starting on the Day of Surgery Reduced Pain Behavior and Neuronal Hyperexcitability Induced by DRG Inflammation**

Next, we investigated the effect of oral eplerenone administered starting at the time of DRG inflammation surgery. Results showed significantly improved withdrawal threshold to von Frey filament testing starting from POD 3 (fig. 3A) and reduced light stroke and acetone responses starting from POD7 (fig. 3, B and C).

Dorsal root ganglia inflammation has previously been shown to increase excitability of neurons in the inflamed ganglion, primarily in myelinated neurons.\textsuperscript{17,19,21} We examined the effects of systemic eplerenone on hyperexcitability of DRG neurons on POD7, when behavioral effects of oral eplerenone were readily observed. In all experiments reported in this study, few differences were observed in cells with action potential duration greater than 1.5 ms (C-cells), and all data presented in the figures are from cells with action potential duration less than 1.5 ms (predominantly myelinated cells; see Electrophysiological Recording under Materials and Methods). In animals treated with oral eplerenone, the spontaneous activity induced by DRG inflammation was reduced to a level not significantly different from that seen in normal (fig. 3D). Eplerenone also significantly reduced hyperexcitability as measured by rheobase and the threshold (fig. 3, E and F).

**Locally Applied Selective GR Agonist Fluticasone Was Very Effective in Reducing Pain Behaviors and Excitability after DRG Inflammation**

We next examined the effect of the highly selective GR agonist fluticasone applied locally to the inflamed DRG. We hypothesized that steroids that activate both the MR and GR receptor would be less effective in the DRG inflammation model than highly GR-selective drugs. Fluticasone does not activate the MR in vitro; instead it acts as a partial antagonist.\textsuperscript{27} When applied locally to the inflamed DRG, fluticasone markedly decreased mechanical hypersensitivity throughout the testing period (fig. 4A). It also reduced withdrawal responses to light stroking touch starting from POD7 and responses to acetone starting from POD3 (fig. 4, B and C).

We also measured excitability of the DRG neurons on POD3, when behavioral sensitivity and spontaneous activity...
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are relatively high. The spontaneous activity in fluticasone-treated animals was reduced to a level not significantly different from normal (fig. 4D). As shown in figure 4, fluticasone also significantly reduced hyperexcitability as measured by the rheobase and threshold (fig. 4, E and F).

**Locally Applied GR/MR Agonist 6-α Methylprednisolone Was Less Effective in Reducing Pain Behaviors and Excitability after Local DRG Inflammation**

We next examined the effects of 6-α methylprednisolone, a steroid commonly used in local injections for low back pain, that activates MR and GR with similar potency in vitro.10 The drug was locally applied to the inflamed DRG. This resulted in significantly improved mechanical hypersensitivity starting from POD 1 and lasting through POD 7 (fig. 5A), but the effect was not as large or long lasting as that of the GR-selective fluticasone (see next section). 6-α Methylprednisolone also reduced responses to light brush strokes and acetone at some time points (fig. 5, B and C).

Because the electrophysiological experiments were conducted in younger females, although behavioral experiments presented in figures 2 through 6 were conducted in older males, we also examined the effects of 6-α methylprednisolone in younger females such as were used in electrophysiological experiments. As shown in Supplemental Digital Content 1, http://links.lww.com/ALN/B50, 6-α methylprednisolone was also only partially effective in reducing mechanical hypersensitivity in females.

We also measured the excitability of the DRG neurons on POD3 in isolated DRGs from 6-α methylprednisolone–treated animals. The inflammation-induced spontaneous activity in animals with 6-α methylprednisolone was reduced, but this effect did not quite reach significance (fig. 5D; P = 0.10). As shown in figure 5, 6-α methylprednisolone significantly reduced hyperexcitability as measured by the threshold, but the normalization of rheobase was not statistically significant (fig. 5, E and F).

**Local Eplerenone Enhanced Effectiveness of 6-α Methylprednisolone in Reducing Pain Behaviors after Local DRG Inflammation**

Finally, we examined the effect of local application to the inflamed DRG of the GR/MR agonist 6-α methylprednisolone combined with the MR antagonist eplerenone. This

Fig. 3. Effect of oral eplerenone (EPL) starting from the day of surgery on pain behaviors and on sensory neuron excitability in locally inflamed dorsal root ganglia (LID) measured on postoperative day (POD) 7. Oral EPL treatment (100 mg kg⁻¹ day⁻¹ starting at the time of surgery and continuing throughout the experiment) significantly reduced the mechanical hypersensitivity induced by dorsal root ganglion inflammation starting at day 3 (A). EPL also reduced responses to light brush strokes and acetone (B, C). *P < 0.05; **P < 0.01; ***P < 0.001, significant effect of oral EPL on that day (two-way repeated-measures ANOVA with Bonferroni post-test). N = 10 animals per group. (D–F) Effects of oral EPL on sensory neuron hyperexcitability. Cells were recorded in the isolated whole dorsal root ganglion preparation, 7 days after inflammation. Several measures of excitability, including percentage of cells showing spontaneous activity (SA; D), rheobase (E), and threshold (F), were increased by LID and ameliorated by systemic EPL. N = 80 normal cells from three animals, 180 LID (POD7) cells from five animals, and 220 LID + oral EPL cells from eight animals. Data in this and subsequent figures are from cells with action potential duration <1.5 ms, which consists almost entirely of cells with myelinated axons. ***P < 0.001, significant difference between normal and LID groups; #P < 0.05; ###P < 0.001, significant difference between LID and LID + oral EPL groups (one-way ANOVA with Bonferroni post-test or Kruskal–Wallis test with Dunn multiple comparison post-test).

PWT = paw withdrawal threshold.
A combination significantly improved mechanical withdrawal threshold at all time points (fig. 6A), almost as effectively as the GR-selective fluticasone. Responses to light brush strokes and acetone were also reduced (fig. 6, B and C).

We also examined the excitability of the DRG neurons on POD3. The spontaneous activity in animals treated with the combined eplerenone and 6-α methylprednisolone was reduced to a level not significantly different from that in normal (fig. 6D). 6-α Methylprednisolone plus eplerenone did not significantly affect the rheobase (fig. 6E), but did significantly reduce hyperexcitability as measured by the threshold in the inflamed DRG neurons (fig. 6F).

For comparison, the mechanical hypersensitivity data from the previous three figures are combined in figure 7. Each of these experiments had its own group of LID (no drug) animals; these groups did not differ from one another at any time point (two-way repeated-measures ANOVA) and those data have been combined. We compared the three different drug groups with this combined control group, using a post-test to compare each group to every other group at all time points. This showed that the highly GR-selective agonist (fluticasone, blue) gives the largest reduction in hypersensitivity. GR/MR agonist 6-α methylprednisolone (gray) gives a more modest effect and its effect is larger when MR effects are simultaneously blocked with eplerenone (green).

Steroid Treatments Had Few Effects in C-cells

As noted above, few significant electrophysiological effects of the steroid treatments presented in figures 3 to 6 were observed when the analysis was applied to cells with action potential durations greater than 1.5 ms, a criterion which our previous work has shown captures 97 to 98% of all C-cells both before and after DRG inflammation. Specifically, for the experiment shown in figure 3, the threshold measured in C-cells on POD7 was significantly more positive (close to the normal value) after systemic eplerenone treatment (n = 64) than in C-cells from inflamed DRG on POD7 (n = 35), and there were no significant differences in spontaneous activity or rheobase. For the experiment shown in figure 4, when compared with C-cells from inflamed DRG on POD3 (n = 18), fluticasone-treated C-cells (n = 12) did not have any significant differences in threshold, spontaneous...
activity, or rheobase. A similar lack of steroid effect on threshold, spontaneous activity, or rheobase was observed for the experiment shown in figure 5 for 6-α methylprednisolone–treated C-cells (n = 18) and for the experiment shown in figure 6 for C-cells (n = 36) from animals treated with a combination of 6-α methylprednisolone and eplerenone.

**Discussion**

Inflammation plays an important role in the pathophysiology of low back pain. Many clinically used steroids activate not only the target GR but also the proinflammatory MR in vitro. We hypothesize that the activation of the MR during DRG inflammation by some clinically used steroids may counteract beneficial effects of GR activation. Consistent with this hypothesis, we found that the MR-selective antagonist eplerenone given orally could reduce mechanical hypersensitivity and sensory neuron hyperexcitability induced by local inflammation of the L5 DRG. The oral route was tested because this drug is currently approved for systemic use in hypertension and heart failure, not for local injection. However, the effects of oral eplerenone on pain behavior were similar to those observed in our previous study using local eplerenone application to the inflamed DRG, and our electrophysiological data showed that systemic eplerenone decreased the spontaneous activity of DRG neurons on POD7, so it is feasible that the effects of systemic eplerenone are primarily or entirely due to its effects on the DRG. Systemic eplerenone could also have antinociceptive effects when given after pain was established (day 7), an experiment also more relevant to the clinical situation.

Also consistent with our hypothesis, the very selective GR agonist fluticasone was highly effective in reducing mechanical pain behaviors when applied locally to the inflamed DRG. This drug is unable to activate the MR in vitro, in contrast to many clinically used steroids. Fluticasone is actually a partial antagonist at the MR receptor as well as a direct enhancer of potassium channel activity; however, the latter effect occurs at concentrations orders of magnitude higher than GR activation. Fluticasone is currently used topically and as an inhaled steroid for asthma but is not formulated for epidural injections. In contrast, 6-α methylprednisolone, commonly used for local injections for low back pain, activates the MR and GR with almost equal potency in vitro. In our study,
this drug applied locally to the DRG reduced mechanical pain behaviors but was significantly less effective than fluticasone. Its effectiveness was improved by combining it with the MR blocker eplerenone. Taken together, these behavioral data suggest that MR blockade and/or use of more GR-selective corticosteroids might be useful in improving steroid response in low back pain conditions involving inflammation.

Our characterization of steroids as GR selective or as dual MR/GR agonists relied on published in vitro studies using cell lines with reporter constructs, in which a reporter gene is placed downstream from regulatory binding sites to which the activated GR or MR transcription factors bind and activate transcription (transactivation).9,10 However, such experiments may not fully describe how a drug will act in vivo. Many anti-inflammatory effects of GR agonists are due to transrepression, that is, the GR receptor directly or indirectly repressing expression of genes activated by other, proinflammatory transcription factors, especially nuclear factor κ B.33,34 These genes may lack a GR-binding site in their own promoters. Conflicting results have been obtained evaluating GR agonist effects on nuclear factor κ B repression, depending on the cell system used; and not all steroids used in low back pain have been evaluated.33–35 Steroids may differently affect transrepression and transactivation. The nuclear factor κ B system is expressed in DRG neurons and has been implicated in inflammatory and neuropathic pain.36 In some tissues, the MR can activate the nuclear factor κ B system,37–39 which might contribute to its apparent pronociceptive role in DRG.

Transrepression requires the MR and GR to be expressed within the same cell. Our data are consistent with the applied steroids working locally at the level of the DRG, but do not address the cellular locations of MR and GR receptors. Some cells in the inflamed DRG (e.g., neurons and macrophages) coexpress the two receptors, but our data cannot rule out effects of MR and GR in different cell types contributing to the observed effects.

Mechanisms discussed above are classical genomic, nuclear effects expected to modify message RNA transcription. Our
not activated in remote DRGs, and have been combined (red, no added drug, N = 19). For drugs applied locally to the dorsal root ganglia at the time of dorsal root ganglia inflammation, the glucocorticoid receptor (GR)–selective agonist (fluticasone, blue) gave the largest reduction in hypersensitivity (paw withdrawal threshold [PWT]). 6-α Methylprednisolone (gray), which activates both mineralocorticoid receptor (MR) and GR in vitro, gives a more modest effect and its effect is larger when MR effects are simultaneously blocked with an MR antagonist eplerenone (EPL) (green). Two-way repeated-measures ANOVA with Bonferroni post-test comparing each group with every other group indicated that all points with nonoverlapping SEM bars were significantly different from one another. (B) The data on SA from the three previous figures are combined. ***P < 0.001, significant difference between normal and LID groups; #P < 0.05, significantly different from LID group (chi-square test).

Our previous study showed that DRG inflammation activated MR receptors in sensory neurons, and that MR agonists and antagonists had direct effects on neurons in primary culture. If this occurs in patients with low back pain, adding an MR antagonist might improve the response even to GR-selective steroids. The endogenous activator of the MR in our model is not yet known. The MR was not activated in remote DRGs, ruling out activation by a systemically increased steroid. The concentration of active endogenous glucocorticoids can be locally regulated by the two forms of 11β-hydroxysteroid dehydrogenase: type 1 that reversibly forms these active corticosteroids from their 11-dehydroxy forms, and type 2 that inactivates them. Both type 1 and type 2 enzymes were present in the DRG, and the type 1 was significantly up-regulated 3 days after local DRG inflammation by microarray. The activity of these enzymes also depends on the redox state of the cell, which may change with inflammation. The microarray data also showed that aldosterone synthase was present in the DRG. Hence, either local aldosterone or locally increased corticosterone could plausibly act as endogenous MR activators in locally inflamed DRG. These enzymes present another confounding factor for predicting in vivo effects of clinically used steroids, only some of which metabolized by 11β-hydroxysteroid dehydrogenases.

Some effects of the tested steroids may occur at the level of the sensory neuron. All drugs examined reduced hyperexcitability of predominantly myelinated sensory neurons measured in isolated DRG preparations after inflammation. This complements a previous study in which direct effects of MR agonists and antagonists were observed in small-diameter (presumed primarily nociceptive) neurons in acute primary culture. Several studies suggest that in the LID and other pain models, myelinated sensory neurons may play a key role. Spontaneous activity in these neurons is observed in several pain models and blocking this activity effectively blocks the development of pain behaviors. It is not yet clear how spontaneous activity in these neurons contributes to pain behaviors, but they may be myelinated nociceptors or may cause central sensitization. Because our recordings were made in an acutely isolated whole DRG preparation, the steroids applied in vivo may have acted at the level of the sensory neurons, the surrounding glial cells, and/or by reducing excitatory inputs from immune cells to cause the observed reduction in hyperexcitability.

Many previous studies on the roles of MR and GR in rodent pain models have focused on the spinal cord. GR and MR are expressed in dorsal horn, and the GR is up-regulated in several pain models. Conflictting results have been obtained with intrathecal or epidural application of GR or MR ligands. Some findings are similar to
those reported here: in several studies using other back pain models, GR activation and/or MR inhibition at the spinal cord level were antinociceptive.52–54 In another study using a back pain model, intrathecal prednisolone acetate was antinociceptive55; this is more difficult to interpret because this drug activates MR and GR in vitro with similar potency. In contrast, GR inhibition was antinociceptive in a neuropathic pain model (chronic constriction injury), whereas MR antagonists had no effect.49 In another neuropathic pain model (spinal nerve ligation), spinal GR antagonists reduced pain50 in one study, whereas agonists reduced pain in another study.56 Hence conflicting results cannot be explained on the basis of neuropathic versus back pain models being used. Like other stressors, pain models may increase systemic corticosterone49,52 which should activate both MR and GR. Systemic application of MR- or GR-acting drugs also gives mixed results in rodent pain models: In naive animals, both corticosterone and a specific GR agonist were antinociceptive in long-term treatment.57 Systemic MR antagonists reduced neuropathic but not chemotherapy pain behaviors in one study.58 However, in both a back pain model49 and a neuropathic pain model,60 stress prolonged or enhanced pain behaviors. In contrast, adrenalectomy was antinociceptive and systemic GR agonists were pronicceptive in a different neuropathic pain model.61 These conflicting results may in part depend on the location of MR and GR receptors, or differing levels of stress in different studies.

In conclusion, we have used a preclinical model of low back pain to provide evidence that the MR (aldosterone receptor), which is activated by some clinically used steroids, may be pronicceptive at the level of the sensory ganglia. Manipulating the activity of the MR in the sensory ganglia might improve the effectiveness of local steroid treatments for some forms of low back pain.

Acknowledgments
The authors thank Mark Baccei, Ph.D., Department of Anesthesiology, University of Cincinnati, Cincinnati, Ohio, for comments on this article.

This study was funded by grant nos. NS55860 and NS45594 from the National Institutes of Health, National Institute of Neurological Disorders and Stroke, Bethesda, Maryland (to Dr. Zhang).

Competing Interests
The authors declare no competing interests.

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References


ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

Wood, Camphor, and an Antidote for Avertin Overdoses

When Wood Library-Museum Founder Paul Meyer Wood began searching for ways to reverse the depressing effects of the rectally administered basal anesthetic Avertin (tribromoethanol), one of many antidotes that he investigated was Camphor. Traditionally considered a cardiac and respiratory stimulant, Camphor could be supplied in an oily solution for either intramuscular or subcutaneous administration. The 1cc ampoule depicted (above) was manufactured by Detroit’s Parke, Davis & Company. (Copyright © the American Society of Anesthesiologists, Inc.)

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