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Endogenous Opioids Regulate Expression of Experimental Autoimmune Encephalomyelitis: A New Paradigm for the Treatment of Multiple Sclerosis

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Preclinical investigations utilizing murine experimental autoimmune encephalomyelitis (EAE), as well as clinical observations in patients with multiple sclerosis (MS), may suggest alteration of endogenous opioid systems in MS. In this study we used the opioid antagonist naltrexone (NTX) to invoke a continuous (High Dose NTX, HDN) or intermittent (Low Dose NTX, LDN) opioid receptor blockade in order to elucidate the role of native opioid peptides in EAE. A mouse model of myelin oligodendrocyte glycoprotein (MOG)-induced EAE was employed in conjunction with daily treatment of LDN (0.1 mg/kg, NTX), HDN (10 mg/kg NTX), or vehicle (saline). No differences in neurological status (incidence, severity, disease index), or neuropathological assessment (activated astrocytes, demyelination, neuronal injury), were noted between MOG-induced mice receiving HDN or vehicle. Over 33% of the MOG-treated animals receiving LDN did not exhibit behavioral signs of disease, and the severity and disease index of the LDN-treated mice were markedly reduced from cohorts injected with vehicle. Although all LDN animals demonstrated neuropathological signs of EAE, LDN-treated mice without behavioral signs of disease had markedly lower levels of activated astrocytes and demyelination than LDN- or vehicle-treated animals with disease. These results imply that endogenous opioids, evoked by treatment with LDN and acting in the rebound period from drug exposure, are inhibitory to the onset and progression of EAE, and suggest that clinical studies of LDN are merited in MS and possibly in other autoimmune disorders. Exp Biol Med 234:1383–1392, 2009

Key words: experimental autoimmune encephalomyelitis; multiple sclerosis; naltrexone; opioids; behavior; neuropathology

Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system that affects over 400,000 people in the U.S. and approximately 2 million people worldwide (1, 2). The origin and pathogenesis of this disease remain to be elucidated, and current therapies are not fully effective (3. 4). New avenues of research are needed to understand the biology of MS and to devise safe and efficacious treatment modalities.

The endogenous opioid system is comprised of native opioid peptides and classical and non-classical opioid receptors (5–7). Originally found to be related to neurotransmission (5–7), the endogenous opioid system has a panoply of biological actions, including those associated with immunity (8) and the regulation of cell proliferation (9–12). A number of intriguing clinical observations suggests that endogenous opioids may be involved in MS. Gironi and colleagues (13) have reported a reduction in β-endorphin levels in peripheral blood mononuclear cells (PBMCs) from patients with clinically inactive MS, but demonstrate an increase in β-endorphin in PBMCs from patients experiencing a relapse. Jankovic (14) has found a beneficial effect of enkephalin treatment on patients with chronic severe progressive MS.

Given these provocative findings, the present report was designed to inquire whether endogenous opioids influence the incidence, onset and/or progression of experimental autoimmune encephalomyelitis (EAE), a model that has proven useful in understanding the biology

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and treatment of MS (4, 15). To address this question, our strategy was to utilize naltrexone (NTX), a general opioid receptor antagonist (16) that is devoid of intrinsic activity (5, 17, 18), to block endogenous opioids from opioid receptors either continuously (high dose naltrexone, HDN) or intermittently (low dose naltrexone, LDN). The response to opioid antagonist administration is an upregulation in the production of opioid peptides and opioid receptors (19–22). Unlike continuous opioid receptor blockade wherein the opioids and their receptors do not have an opportunity to interface, pharmacokinetic studies (23) and nociceptive investigations (24, 25) have shown that blockade of endogenous opioids from opioid receptors for a short period of time (4–6 hr) each day provides a window of 18–20 hr wherein the high levels of endogenously produced opioids can interact with the elevated levels of opioid receptors. This intermittent opioid receptor blockade leads to a supersensitive response (e.g., depressed cell proliferation) (26–29) in the interval when the opioid antagonist is no longer present. The effects of opioid antagonists in this technique of elucidating function not only are stereospecific (30, 31), but dependent on the duration of opioid receptor blockade rather than the dosage of drug (25, 31). Thus, the use of opioid antagonists to modulate the endogenous opioid system in order to understand function of opioid-opioid receptor interactions in biological processes and disease has been successful in many studies (9, 11, 24, 25).

In the present study, mice were subjected to injections of myelin oligodendrocyte glycoprotein (MOG) and received daily injections of HDN which rendered a continuous 24 hr opioid receptor blockade, or LDN which invoked an intermittent opioid receptor blockade. Our findings show that intermittent opioid receptor blockade with LDN, but not continuous opioid receptor blockade with HDN, prevents behavioral signs of disease in some animals and markedly attenuates the severity of clinical signs and neuropathology of EAE in others. These results suggest that endogenous opioids are inhibitory trophic factors that in part determine the onset and progression of EAE, offering a new paradigm in understanding the biology of EAE and providing implications for the pathogenesis and treatment of MS.

Materials and Methods

Induction of Experimental Autoimmune Encephalomyelitis. C57BL/6 female mice (7–10 weeks) were purchased from Harlan Laboratories (Indianapolis, IN) and maintained at the Penn State Hershey Medical Center, with food and water provided *ad libitum*. All experiments were conducted in accordance with the NIH guidelines on animal care and were approved by the Penn State Hershey Institutional Animal Care and Use Committee.

Mice were given 900 μg of mouse $MOG_{35–55}$ (Penn State College of Medicine Core Facility, 99% purity) dissolved in phosphate-buffered saline (PBS) and emulsified in complete Freund's adjuvant (CFA, DIFCO Laboratories, Lawrence, KS), supplemented with 500 μg heat-inactivated *Mycobacterium tuberculosis* (DIFCO Laboratories) through a series of injections as modified from Suen *et al*. (32). The intramuscular injections were divided equally between the left and right flanks. Intraperitoneal injections of 500 ng pertussis toxin (List Biological Laboratory, Campbell, CA) dissolved in 200 μl PBS were administered on days 0 and 2.

Drug Treatment. Beginning on the first day of MOG injections, NTX (Sigma Chemicals, St. Louis, MO) was dissolved in 200 μl of PBS for a final dose of either 0.1 mg/kg (LDN) or 10 mg/kg (HDN) NTX, and was injected daily (intraperitoneal) between 1300 and 1600 hr. These groups were termed MOG+LDN and MOG+HDN, respectively. MOG inoculated animals receiving daily injections of an equivalent volume of PBS comprised the MOG+Vehicle group. Animals injected with CFA, PBS, *M. tuberculosis* and pertussis, and treated daily with PBS served as Control+Vehicle animals. Some animals that were not given CFA, pertussis toxin or MOG peptide were included for study as Normals. At least 15 animals/group, representing 4 independent experiments, were utilized.

Behavioral Assessment. Following the encephalitogenic challenge, mice were observed daily for up to 30 days and clinical manifestations of EAE were scored by an observer masked to the identity of the treatment groups. The EAE scoring system followed Suen *et al*. (32) and Encinas *et al*. (33), with disease severity evaluated as follows: 0 = no clinical symptoms; 1 = loss of tail tonicity; 2 = wobbly gait; 2.5 = single hind limb paralysis; 3 = complete hindlimb paralysis; 4 = paralysis of four limbs; 5 = death. The animals had to display EAE signs on two consecutive days in order to receive a positive score. Mean maximal severity score was calculated according to Milicevic *et al*. (34) and represented the mean of the maximal disease score that each mouse in a group developed over the course of the experiment.

A disease index was calculated according to Suen *et al*. (32) by adding daily average disease scores over 30 days, dividing the sum by the average day of disease onset for each group, and multiplying this result by 100. For animals that did not display symptoms of EAE, the day of disease onset was assigned as the day after the experiment was terminated. The maximum average disease score for each group, and the day on which it occurred, were noted from the data on the disease index.

Neuropathology. At 20 days of treatment, mice were anesthetized with a cocktail (0.1 ml) of ketamine (30 mg/kg), xylazine (5 mg/kg) and acepromazine (2 mg/kg), and perfused transcardially with 10% neutral buffered formalin. Tissues were fixed for 48 hr, and spinal cords dissected. Matched paraffin sections (10 μm) of the lumbar region (L4–L6) (35) were stained for (i) myelin with Luxol Fast Blue (Roboz Surgical Instrument Co., Washington, DC)-Cresyl Violet (Sigma, St. Louis, MO), (ii) activated astrocytes (stellate-shaped cells with hypertrophic processes) with a polyclonal antibody to GFAP (1:500 dilution,

DAKO, Carpinteria, CA), and (iii) neuronal damage as indicated by the presence of non-phosphorylated neurofilament H protein using a mouse monoclonal antibody to SMI-32 (1:500 dilution, Covance, Princeton, NJ). Controls for antibody staining included sections stained with secondary antibody only. At least two sections/animal, with 6–9 animals/group, were evaluated for each measure.

Demyelination. Quantification of demyelination was modified from the methods by Tsundoa *et al.* (36) and MacNamara *et al.* (37). Data were expressed as the percentage of mice with at least one demyelinated quadrant.

Astrocyte Activation. Following the methodology of Stichel and Luebber (38), Ayers *et al.* (39) and Bannerman *et al.* (40), the number of reactive astrocytes in a grid measuring 0.053 mm^2 observed at ×400 in each section of the lumbar spinal cord was determined. Data were expressed as the number of activated astrocytes/mm^2.

Neuronal Damage. The number of cells stained for SMI-32 was counted in each section (41).

Statistics. The percentage of mice scored for a particular behavior, as well as data on the percentage of mice with demyelinated quadrants, were analyzed by non-parametric analysis using Chi-square tests. All other data were analyzed with one-way analysis of variance (ANOVA), with subsequent comparisons made with Newman-Keuls (GraphPad Prism, La Jolla, CA). P values less than 0.05 were considered statistically significant.

Results

General Observations. No animal displayed lesions or ulcerations at the injection site(s), and no behavioral abnormalities were noted in any group within 1 week after the injection. No changes in body weight were recorded between groups, and no deaths were recorded in any group. The Normal group of mice did not display abnormal behavior or neuropathological signs.

High Dose Naltrexone and Neurological Status. Signs of clinical disease were first observed for MOG+Vehicle and MOG+HDN groups on days 12 and 13, respectively, and the average day of the onset was 16.2 ± 0.4 and 16.6 ± 0.6, respectively. All mice in the MOG+Vehicle group had behavioral signs of EAE by day 22, whereas animals in the MOG+HDN group did not reach 100% disease incidence until day 25 (Fig. 1A). All animals in the MOG+HDN group exhibited disease signs through day 30 (Fig. 1B), as did all but one animal in the MOG+Vehicle group that reverted from a score of 1 to 0 on day 29. The mean maximal severity scores for the MOG+Vehicle and MOG+HDN groups were comparable (i.e., 2.0 ± 0.1 and 2.1 ± 0.1, respectively). Examination of the distribution of disease score on day 20 revealed no differences in the percentage of animals demonstrating scores of 0, 1, 2 or 3 (Fig. 1C). The maximum average disease score was 2.0 for the MOG+Vehicle and MOG+HDN groups; however, the day of maximum average disease score was 24–27 for the MOG+Vehicle group, and 21–22 for the MOG+HDN group. Calculation of the disease index revealed no difference between the MOG+Vehicle and MOG+HDN groups (Fig. 1D).

High Dose Naltrexone and Neuropathology. The number of activated astrocytes for the MOG+Vehicle and MOG+HDN groups did not differ, but was approximately 11-fold greater than that for the Control+Vehicle group (Fig. 2A). No demyelination was recorded in the Control+Vehicle group, whereas the percentage of mice with demyelinated quadrants was 38% and 50% for the MOG+Vehicle and MOG+HDN groups, respectively (Fig. 2B). However, the MOG+Vehicle and MOG+HDN groups had 5.9- and 6.7-fold greater numbers of SMI-32 neurons/section than in the Control+Vehicle group (2.3 ± 0.6) (Fig. 2C).

Low Dose Naltrexone and Neurological Status. The earliest day of behavioral signs of disease for both the MOG+Vehicle and MOG+LDN groups was day 12. The average day of disease onset was 16.1 ± 0.5 and 21.9 ± 1.2 for the MOG+Vehicle and MOG+LDN groups, respectively; these differences were statistically significant ($P < 0.001$). All mice in the MOG+Vehicle group displayed behavioral signs of EAE by day 22, whereas only 66% of the animals in the MOG+LDN group showed clinical signs of disease. In fact, on days 20, 25 and 30, there was a significant reduction in the number of MOG+LDN mice in contrast to those in the MOG+Vehicle group that displayed EAE (Fig. 3A). Mice in both the MOG+Vehicle and MOG+LDN groups displayed a similar course of disease, but the severity of disease for the MOG+LDN group was significantly reduced from days 16 through 30 relative to the MOG+Vehicle group (Fig. 3B). The mean maximal severity scores for the MOG+Vehicle and MOG+LDN groups were 2.0 ± 0.1 and 1.6 ± 0.2, respectively. Examination of the distribution of disease score on day 20 revealed significant differences in the percentage of animals demonstrating scores of 0 and 2 (Fig. 3C). Of the animals in the MOG+LDN group, 55% were free of disease on day 20, while only 9% of the animals in the MOG+Vehicle group did not display disease. Furthermore, only 28% of the animals in the MOG+LDN group had a wobbly gait (i.e., score of 2) compared to 73% of the mice in MOG+Vehicle group. The maximum average disease score was 2.0 on day 24 for mice in the MOG+Vehicle group, but 1.2 on day 30 for subjects in the MOG+LDN group. Calculation of the disease index revealed a 2.5-fold reduction in the MOG+LDN group of animals compared to the MOG+Vehicle cohort (Fig. 3D).

Low Dose Naltrexone and Neuropathology. The numbers of activated astrocytes in the spinal cord of mice in the MOG+Vehicle, MOG+LDN that did not have disease (EAE^-) and MOG+LDN that exhibited clinical signs (EAE^+) groups were 10.2-, 8.1- and 3.7-fold, respectively, greater than that for the Control+Vehicle group (Fig. 4A). However, the MOG+LDN/EAE^- group had markedly fewer

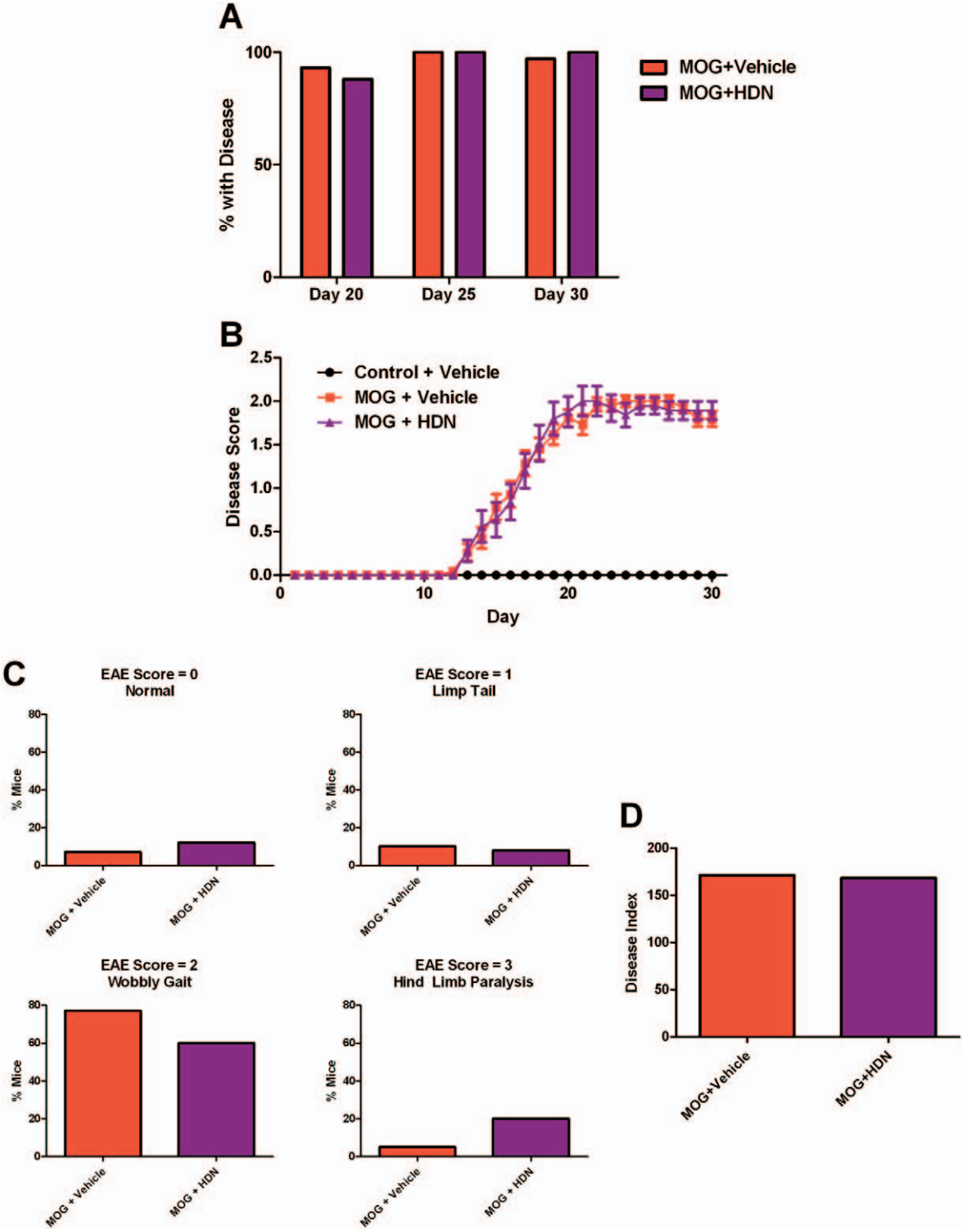

Figure 1. MOG-treated mice receiving HDN or Vehicle developed sustained chronic disease. *n* values for MOG+HDN, MOG+Vehicle and Control+Vehicle groups ranged from 19–25, 30–42 and 15–25, respectively. (A) The incidence of mice assigned a disease score $\geq$1 on days 20, 25 and 30. (B) The daily mean score of disease for the MOG+HDN and MOG+Vehicle groups. No animals in the Control+Vehicle group exhibited behavioral signs of disease. Data represent means $\pm$ SE. (C) Distribution of disease score on day 20. (D) Disease Index calculated for 30 days after the initial MOG injection. A color version of this figure is available in the online journal.

activated astrocytes compared to the MOG+Vehicle and MOG+LDN/EAE$^+$ groups. No demyelination was recorded in the Control+Vehicle group, whereas the percentages of mice with demyelinated quadrants were 33%, 67% and 14% for the MOG+Vehicle, MOG+LDN/EAE$^+$ and MOG+LDN/EAE$^-$ groups, respectively (Fig. 4B); the only significant difference between groups occurred for a significant elevation for the MOG+LDN/EAE$^+$ group from the Control+Vehicle group. A small number (1.7 ± 0.5) of neurons in the lumbar region of the spinal cord of animals in the Control+Vehicle group exhibited SMI-32 positivity, a sign of neuronal damage. MOG+Vehicle, MOG+LDN/EAE$^+$ and MOG+LDN/EAE$^-$ groups had 3.1- to 7.3-fold greater number of SMI-32 neurons relative to the Control+Vehicle group (Fig. 4C); however, only the MOG+Vehicle and MOG+LDN/EAE$^-$ mice were significantly increased in the number of damaged neurons from the Control+Vehicle group. Animals in both the MOG+LDN/EAE$^+$ and the MOG+LDN/EAE$^-$ groups had significantly fewer SMI-32 neurons in the spinal cord relative to that of the MOG+Vehicle.

Discussion

The results of this study suggest for the first time that endogenous opioid peptides interfere with the autoimmune encephalitogenic process, and influence the onset and progression of EAE. Intermittent opioid receptor blockade using daily injections of LDN, at a dosage of NTX that was ~0.01% of the LD_{50} and without toxicological symptoms (42), had a remarkable neuroprotective effect on MOG-injected animals. Several lines of evidence support this conclusion. First, over one-third of the MOG-injected animals receiving LDN did not express any neurological signs of EAE in comparison to 100% of the MOG+Vehicle mice. Second, of those LDN-treated mice with disease on day 20, the severity of EAE was substantially reduced. Third, the disease index, mean maximal severity scores and maximum average disease scores were markedly reduced, and the average day of disease onset notably increased, in the MOG+LDN group in contrast to MOG+Vehicle subjects. However, it is important to note that mice in the MOG+LDN group, whether exhibiting behavioral signs of disease or not, did not escape at least some neuropathological damage. Of course, the present study has focused on only a short window in terms of the effects of LDN on EAE, and further investigations across a longer period of time are

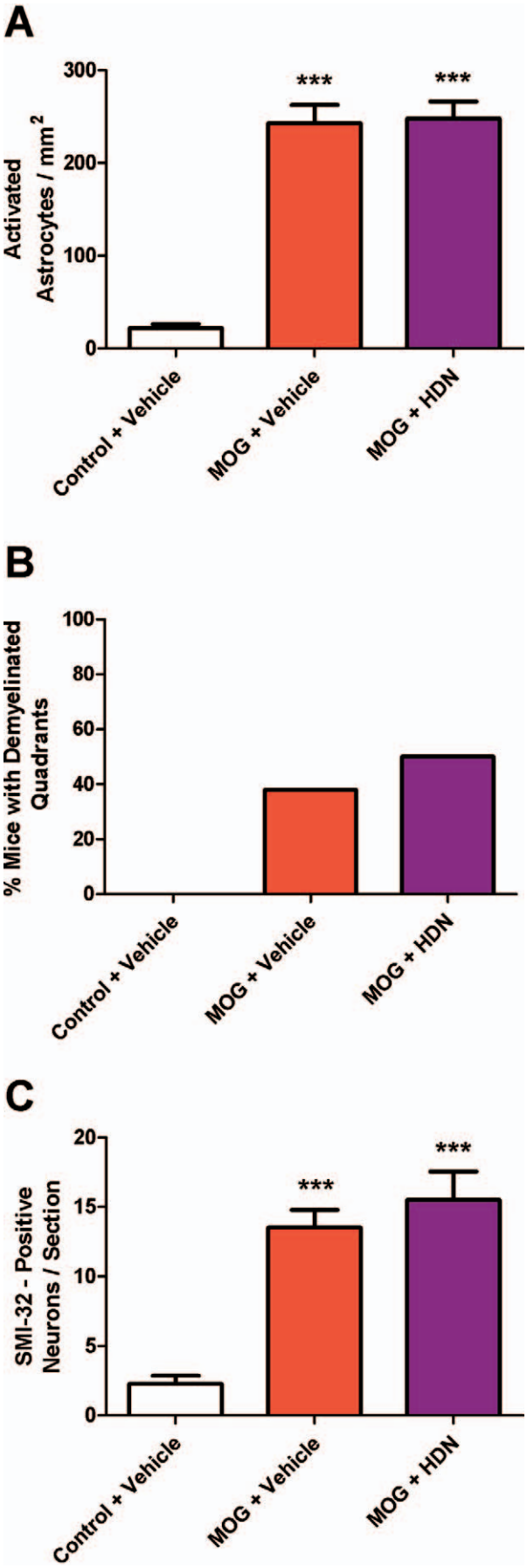

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Figure 2. MOG-treated mice receiving HDN or Vehicle, as well as animals in the Control+Vehicle group, exhibited signs of neuropathology in the lumbar region of the spinal cord. The number of activated astrocytes (A) and damaged neurons (C) is presented as means ± SE. Demyelination is presented as the percent of mice in a group with at least one spinal cord quadrant with demyelination (B). Significantly different from Control+Vehicle at $P < 0.001$ (***). A color version of this figure is available in the online journal.

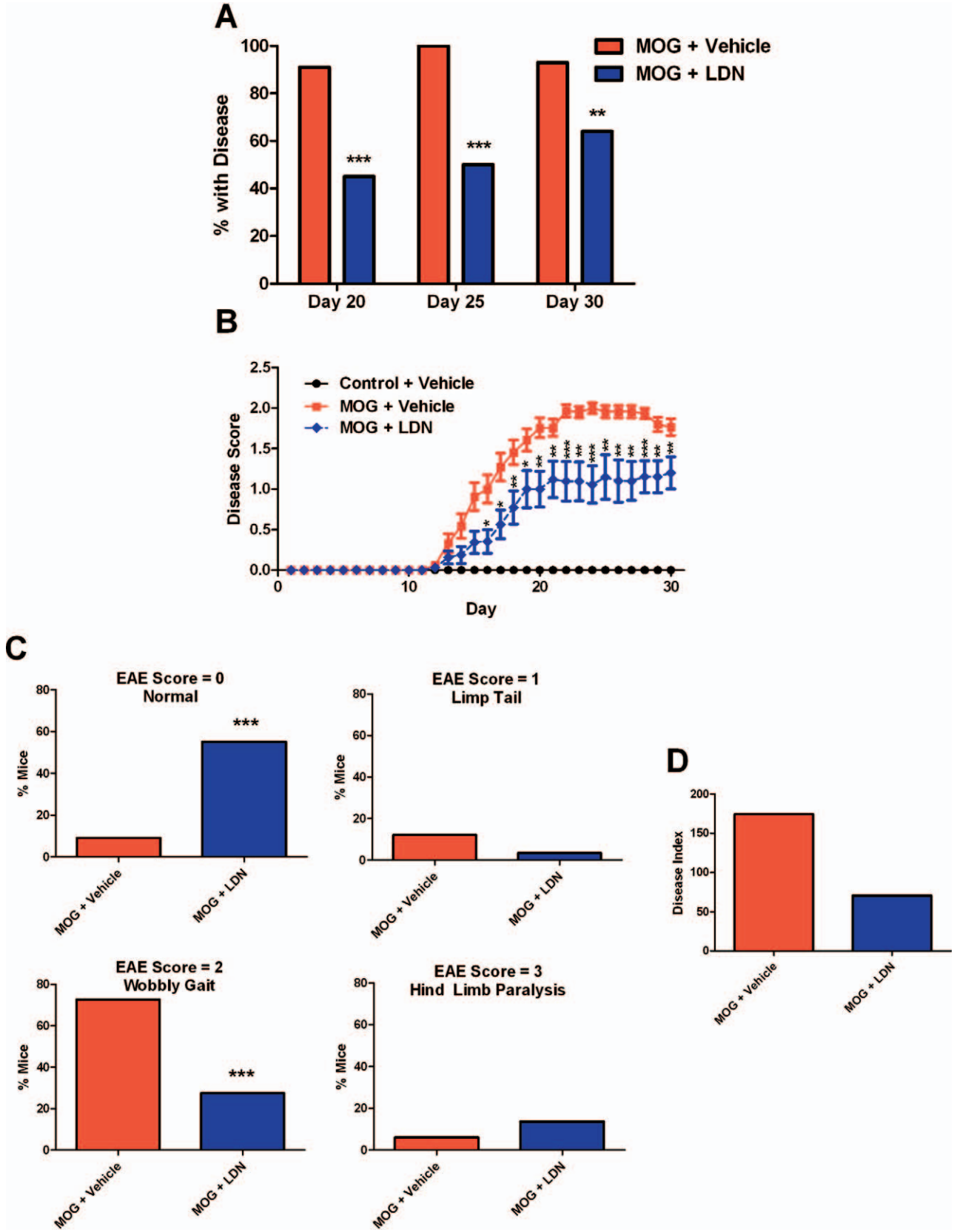
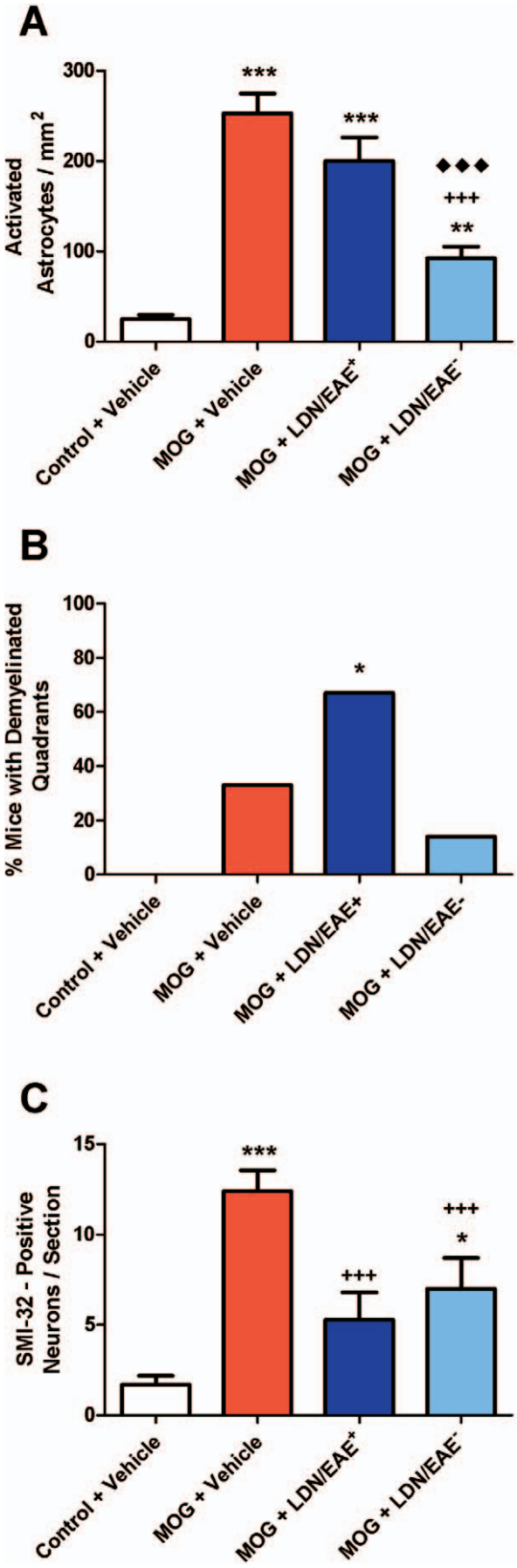


Figure 3. The profile of disease in MOG-injected mice. (A) The incidence of mice assigned a disease score ≥ 1 on days 20, 25 and 30. (B) The daily mean score of disease for the MOG+LDN ($n = 26$–29) and MOG+Vehicle ($n = 30$–33) groups. No animals in the Control+Vehicle group ($n = 15$) exhibited behavioral signs of disease. Data represent means ± SE. Significantly different from MOG+Vehicle at $P < 0.05$ (★), $P < 0.01$ (★★) or $P < 0.001$ (★★★). (C) Distribution of disease score on day 20. (D) Disease Index calculated for 30 days after the initial MOG injection. A color version of this figure is available in the online journal.



required to understand the full temporal-spatial sequence of events related to LDN and EAE. Evaluation of other regions of the spinal cord (e.g., cervical), as well as the brain, in mice subjected to MOG and treated with LDN also is needed to provide a more complete picture of drug action with respect to correlating neuropathology and disease. Moreover, whether manipulation of endogenous opioid systems by LDN initiated in animals with established disease, or prior to induction of EAE, will reduce clinical signs and/or neuropathology of EAE warrants investigation.

Using a model of complete opioid receptor blockade with the opioid antagonist NTX, we found that HDN had no effect on the onset, incidence or severity of clinical signs of EAE. Moreover, neuropathological examination of the lumbar region of the spinal cord in mice receiving HDN revealed that the number of activated astrocytes and damaged neurons, as well as the magnitude of demyelination, did not differ from mice subjected to MOG that received daily injections of vehicle. Because blockade of opioid peptides did not influence the course of disease, these results could suggest endogenous opioids are not tonically active with respect to EAE. Alternatively, the temporal window of the course of EAE expression utilized in these experiments may have been so short as to obfuscate any differences that may have occurred. Further studies using other models of EAE are needed to address this question, particularly since endogenous opioid action often is a tonically active process (43).

The present results are consistent with previous data indicating that opioid peptides are associated with EAE and MS. With respect to animal studies with EAE, Jankovic and Maric (44) reported that rats with EAE and receiving injections of [Met^5]-enkephalin, experienced a prevention or delay in paralysis. However, only 10% to 70% of the saline-treated rats presented signs of disease; therefore, the induction method may have introduced a confounding variable making interpretation of the data difficult. In humans with MS, β-endorphin (13), but not dynorphin (45) levels were increased in patients with clinical relapse. Jankovic (14), utilizing a small population of patients, has reported that treatment with [Met^5]-enkephalin had a beneficial effect on subjects with chronic severe progressive MS. Our investigation takes a totally different strategy by

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Figure 4. MOG-treated mice receiving LDN or Vehicle, as well as animals in the Control+Vehicle group, exhibited signs of neuropathology in the lumbar region of the spinal cord. MOG injected mice receiving LDN were divided into groups of animals that did (MOG+LDN/EAE$^+$) or did not (MOG+LDN/EAE$^-$) express behavioral signs of disease. The number of activated astrocytes (A) and damaged neurons (C) is presented as means ± SE. Demyelination is presented as the percent of mice in a group with at least one spinal cord quadrant with demyelination (B). Significantly different from Control+Vehicle at $P < 0.05$ (★★), $P < 0.01$ (★★) or $P < 0.001$ (★★★). Significantly different from MOG+Vehicle at $P < 0.001$ (+++). Significantly different from MOG+LDN/EAE$^+$ $P < 0.001$ (◆◆◆). A color version of this figure is available in the online journal.

examining the repercussions of different opioid antagonist regimens on EAE. The data document that one or more opioid peptides are important to the initial phase of pathogenesis as well as to the progression of disease. These results also may have meaning in the clinical realm, suggesting that continuous opioid receptor blockade (e.g., high doses or multiple daily exposures to LDN) is not efficacious in treating disease and even could have adverse outcomes. Further studies are needed to explicate the opioid peptide(s) and receptor(s) involved with EAE, and possibly with MS.

Women in pregnancy, a period when there is a high level of endogenous opioids (46), experience remission of MS and have fewer relapses during their pregnancy. However, these women exhibit a marked increase in relapse rate 3 months after delivery, when endogenous opioid levels are decreased (4, 47). Given the present results, this finding could be interpreted that one or more of the elevated opioids elicited during pregnancy are acting with relevant receptors to attenuate overt expression of MS. Moreover, once these high levels are reduced to basal values following delivery, there is a loss of the positive aspects provided by opioid-receptor interactions and an exaggeration of disease.

Proteases associated with degradation of opioid peptides are increased with MS. For example, Ziaber *et al*. (48, 49) reported an increase in CD10 (neutral endopeptidase-NEP, EC 3.4.24.11) and CD13 (aminopeptidase N, AP-N, EC 3.4.11.2) in MS patients during the course of exacerbation and chronic MS, but low expression of these molecules during remission. Because these ectoenzymes are enkephalin-degrading (50, 51), this could depress the population of enkephalins related to the inhibition of cell proliferation. If this is the case, T cell proliferation could be increased, exacerbating the development of MS. Thus, our findings offer an explanation for these observations related to enkephalinases and MS by providing evidence that these opioid peptides and receptors may be a determinant of the course of MS.

The mechanism involved with the attenuation of EAE by LDN is unclear. LDN has been found to repress development and neoplasia by modulating the endogenous opioid systems (11, 12, 24, 25, 30). Research has shown that LDN produces a short window of opioid receptor blockade that elicits a compensatory rise in opioid peptides and opioid receptors and, in the interval when LDN is no longer blocking opioid receptors, the elevated levels of opioids and opioid receptors lead to a supersensitive functional response. Subsequent studies (25, 31) have demonstrated that it is the duration of opioid receptor blockade that was a key element in LDN action, and that LDN was having an indirect effect on cell proliferation by manipulating an opioid peptide (i.e., OGF) and receptor (i.e., OGFr). In the case of development and neoplasia, cell proliferation is depressed. Cheng and colleagues (52–55) have shown that OGFr is associated with a nuclear localization-dependent shuttling of the OGF-OGFr complex from the cytoplasm to the nucleus wherein there is an increase in the cyclin dependent kinase inhibitory pathway leading to delays in the G_1-S phase of the cell cycle. With respect to the present investigation, there was a dose-dependent response, with the lower (LDN) but not the higher (HDN) dosage altering the course of EAE. These results bear striking similarity to the data reported earlier for the effects of LDN in development and cancer. If, indeed, LDN is directed towards diminishing cell proliferative events by way of the OGF-OGFr pathway, it may be that a delay in the G_1-S phase of the cell cycle with regard to relevant cells (e.g., autoreactive T cells) could downregulate downstream events that would lead to a suppression of the behavioral and morphological repercussions of EAE. Further study is needed to resolve whether the mechanism of LDN in influencing the course of EAE involves the OGF-OGFr axis and/or other opioid peptides and receptors (μ, δ, κ).

It is unknown whether these results can be extrapolated to other models of EAE or to human subjects with MS. If our findings with the EAE model in mice are meaningful to MS, the clinical implications of our results are considerable. NTX is used in the treatment of drug and alcohol abuse at dosages of 50 mg, while the recommended dosages of LDN are 3 to 10 mg/day (U.S. Patent 4,689,332). LDN already has been successful in the treatment of Crohn's disease (56), and a dosage of 4.5 mg/day proved safe and efficacious. With respect to MS, Gironi and colleagues (57) have recently reported a pilot trial of LDN in patients with primary progressive MS, and found LDN safe and well tolerated. If LDN is found to have efficacy in attenuating MS, it would represent a safe, non-toxic agent that is generic and available in oral preparation. This would have the exciting outcome of offering patients an alternative to costly, invasive/injectable medications that frequently have toxic side-effects. Based on findings in this study, clinical studies are merited that would not only investigate LDN in patients with established MS, but also in patients presenting with clinically isolated syndrome. Moreover, if our results with EAE can be extended to other autoimmune diseases (e.g., Lupus, Crohn's disease), recruitment of endogenous opioids and opioid receptors by modulation using LDN may be of clinical importance.

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