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Corticosterone regulates expression of CCL2 in the intact and chemically injured hippocampus

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Abstract

Expression of the chemokine (C–C motif) ligand 2 (CCL2), also known as, monocyte chemoattractant protein (MCP)-1, increases in response to disease-, trauma-, or toxicant-induced damage to the central nervous system (CNS). In the periphery, endogenous and exogenous glucocorticoids are known to suppress CCL2 expression associated with inflammatory conditions. However, such actions of glucocorticoids on CCL2 expression in the CNS remain unknown. Here, we explored the effects of the glucocorticoid, corticosterone (CORT), on the expression of CCL2 and its receptors, CCR2 and CCR5, in the hippocampal formation using intact, adrenalectomized (ADX) and trimethyltin (TMT)-treated rats. An immunosuppressive regimen of CORT did not alter the mRNA expression of CCL2 or its receptors in the hippocampus. ADX, however, markedly increased the expression of CCL2 and CCR2 mRNAs in the hippocampus, while CORT replacement reversed the effects of ADX on CCL2 gene expression. Hippocampal damage resulting from systemic administration of the organometallic neurotoxicant, TMT, was associated with microglial activation, as evidenced by enhanced expression of microglial markers integrin αM (CD11b) and F4/80, as well as, microglia-associated factors, CCL2 and IL-1α. An immunosuppressive dose of CORT, suppressed TMT-induced expression of CCL2. Given the association of CCL2 with microglial activation, it appears that CORT may play a role in regulating microglial activation. However, CORT treatment did not alter TMT-mediated neuronal damage and astrogliosis. Such observations suggest that injury-related expression of microglia-associated chemokines and cytokines may subserve a role unrelated to neuronal damage. In summary, our data indicate that in the CNS, CCL2 gene expression is under negative regulation by glucocorticoids. Published by Elsevier Ireland Ltd.

Keywords: Brain; CCL2; Corticosterone; Glucocorticoids; Microglia; Trimethyltin; MCP-1; Neurodegeneration; Neuroinflammation

Proinflammatory chemokines, of which the β-chemokine, CCL2, is a prominent member, have been shown to be upregulated in association with inflammatory brain processes and diseases, such as multiple sclerosis [8]. More recent evidence has been obtained to link enhanced expression of CCL2 to glial responses associated with traumatic brain injury [2,13]. Additionally, we have found increased expression of CCL2 in association with activated microglia following hippocampal damage caused by systemic administration of the neurotoxic organometal, TMT [6]. While endogenous and exogenous glucocorticoids are known to negatively regulate expression of CCL2 associated with systemic inflammation [5,18] little evidence exists regarding hormonal regulation of CCL2 in the intact or injured brain. Therefore, the purpose of the present study was to determine if the expression of CCL2 and its receptors in hippocampus was regulated by corticosterone (CORT) in the intact, adrenalectomized (ADX) and TMT-exposed rat. The data indicate that CCL2 is regulated by glucocorticoids in the intact and chemically injured hippocampus.

Intact or ADX male Long–Evans rats (6 weeks old; 150–175 g) were obtained from Charles River (Portage, MI) and singly housed in plastic tub cages with aspen shred bedding in a temperature (21 ± 1 °C) and humidity-controlled (50 ± 10%) colony room maintained on a 12-h light:12-h dark schedule. ADX rats were supplemented with 0.85% saline via their drinking water. CORT solutions were prepared in 1.2% ethanol for administration in drinking water. Ethanol vehicle (alone) did not affect any of the measures examined in this study (data not shown). One group of intact rats received either vehicle or 200 μg/ml CORT in drinking water and was sacrificed 5–26 days later. A second group of intact rats received either vehicle or 5 μg/ml CORT in drinking water for 1 week prior to sacrifice. Similarly, one set of ADX rats received either vehicle or 5 μg/ml CORT in drinking water for 1 week prior to sacrifice. A final

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group of intact rats received either vehicle or 200 μg/ml CORT in drinking water for 1 week prior to the administration of vehicle or TMT (8.0 mg/kg, *i.p.*, calculated as the free base, K&K Laboratories, Cleveland, OH). The animals were sacrificed at various intervals of time (1–26 days). Separate control groups were used for each experimental condition and time point evaluated. Thymus and adrenal glands were examined for involution and the gland weights were obtained to record the effectiveness of CORT regimen. The hippocampi from both hemispheres were dissected free-hand; the left hippocampi was immediately frozen in liquid nitrogen for RNA extraction, while the right hippocampi was sonicated in hot (85–95 °C) 1% SDS for assay of GFAP to confirm the expected TMT-induced astrogliosis [1,6]. The tissue samples were stored at −75 °C until analysis. All procedures were performed under protocols approved by the Institutional Animal Care and Use Committee of West Virginia University School of Medicine and the Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health (CDC-NIOSH). The NIOSH animal facility is accredited by the American Association for Accreditation of Laboratory Animal Care.

Total RNA was isolated using the RNeasy Mini kit (Qiagen, Valencia, CA, USA). Total RNA was reverse-transcribed using Superscript™ II RNase H-reverse transcriptase and oligo $(dT)_{12–18}$ primers (Invitrogen, Carlsbad, CA, USA).

The mRNA expression of CCL2, CCR2, CCR5, CD11b (Integrin αM), F4/80 and IL-1α were assayed by real-time PCR analysis (Applied Biosystems, Foster City, CA, USA). PCR amplifications (40 cycles) were performed in a total volume of 50 μl, containing 1 μl cDNA, 300 nM of gene-specific primers or 2.5 μl of appropriate FAM/TAMRA labeled fluorogenic probe/primer set (TaqMan® gene expression assays, Applied Biosystems) and SYBR Green or Universal TaqMan® master mix (Applied Biosystems), respectively. Quantification of gene expression was performed using a standard curve for each gene of interest. The standard curves were linear over 7–9 orders of magnitude. Changes in the levels of mRNA expression were calculated following normalization to the housekeeping genes, ribosomal S29 or GAPDH, and are expressed as percent of control. Separate control groups were used for each experimental condition and time point evaluated.

Total protein in SDS-homogenates was determined by bicinchoninic acid method [14], using bovine serum albumin as standard. GFAP was assayed by a sandwich ELISA method developed in this laboratory [10,11].

The test of significance was performed using one-way analysis of variance (ANOVA) and post hoc group comparisons were made using Dunnett's test. Values were considered statistically significant at 5% level of significance ($P < 0.05$). All results are expressed as the mean ± S.E.M. for each group.

Involution of thymus and adrenal glands serves as an indicator of immunosuppression by exogenous glucocorticoids. Administration of the 200 μg/ml dosage of CORT in drinking water was sufficient to markedly reduce the thymus and adrenal weights within 5 days of treatment (Fig. 1), findings that demonstrate an immunosuppressive effect of this CORT regimen.

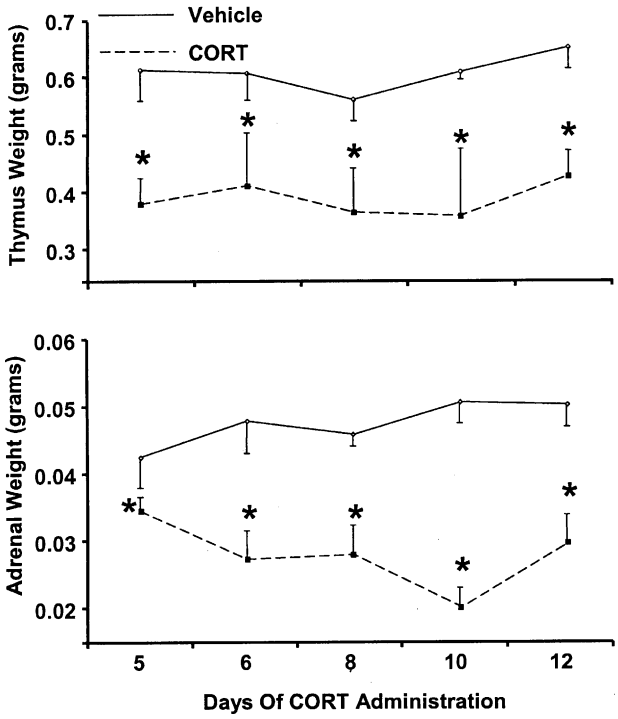

Fig. 1. A high-dose CORT regimen was sufficient to markedly decrease thymus and adrenal weights. Significantly different from corresponding vehicle-treated control ($^{*}P < 0.05$).

High physiological levels of CORT in the drinking water (200 μg/ml) did not affect the expression of CCL2 mRNA in the hippocampus (Fig. 2). CCL2 mRNA levels remained unaltered by CORT supplementation (Fig. 2) over a prolonged duration of time (5–26 days), findings indicative of a lack of regulation by CORT or a maximal suppression from endogenous physiological levels of CORT.

To determine if CCL2 expression was under control of endogenous CORT, ADX rats were subjected to CORT replacement (5 μg/ml) in the drinking water (Fig. 3). CCL2 mRNA levels increased by 12-fold ($P < 0.05$; Fig. 3A) in the ADX group compared to intact controls and CORT replacement prevented this increase (Fig. 3A). These results are consistent with negative

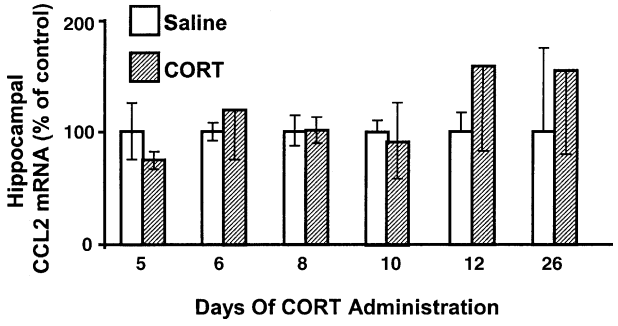

Fig. 2. CCL2 mRNA expression in hippocampus is not affected by an immunosuppressive CORT regimen. Rats were treated with an immunosuppressive dose of CORT (200 μg/ml) in drinking water and sacrificed at 5–26 days post-CORT treatment. Hippocampal mRNA expression of CCL2 mRNA was analyzed by real-time PCR. Values represent the mean ± S.E.M. for five independent observations and are expressed as percent of saline-treated control.

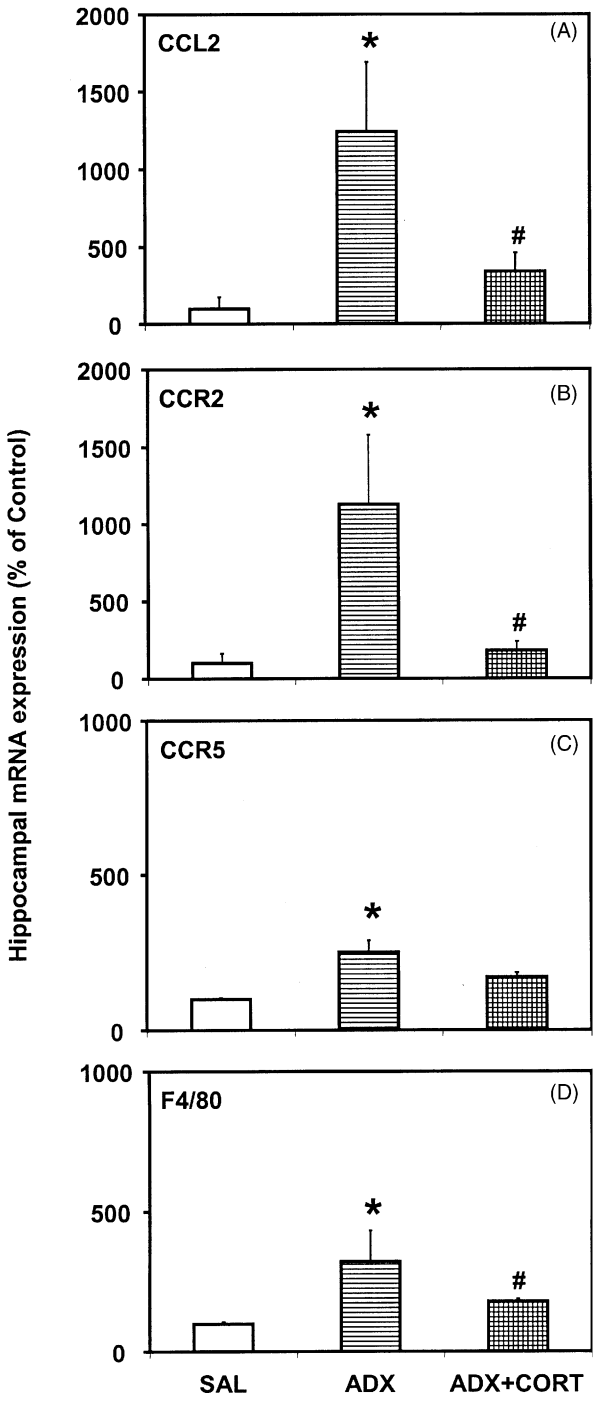

Fig. 3. CCL2, CCR2 and F4/80 mRNA expression were dramatically increased by ADX and these effects were reversed by CORT replacement. ADX rats were administered either vehicle or 5 μg/ml CORT in drinking water and sacrificed 1 week after CORT replacement. Hippocampal mRNA expression of CCL2, CCR2, CCR5 and F4/80 were analyzed by real-time PCR. Values represent the mean ± S.E.M. for five independent observations and are expressed as percent of saline-treated (intact) controls. Significantly different from intact group ($^{*}P<0.05$). Significantly different from ADX group ($^{\#}P<0.05$).

regulation of CCL2 mRNA by CORT and suggest that it is fully suppressed at physiological CORT levels (compare with results for Fig. 5). The effects of ADX and CORT replacement therapy on the gene expression of the CCL2 receptors, CCR2 and

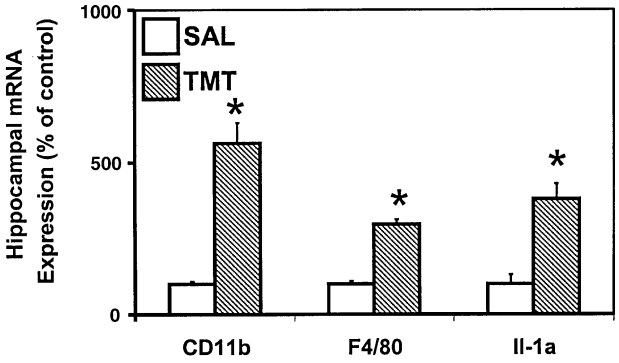

Fig. 4. Hippocampal injury due to administration of TMT is associated with microglial activation. Rats were treated with TMT (8 mg/kg, *i.p.*) and sacrificed on post-dosing day 5. Hippocampal mRNA expression of microglial markers and microglia-derived factors was analyzed real-time PCR. Values represent the mean ± S.E.M. for five independent observations and are expressed as percent of vehicle-treated controls. Significantly different from saline-treated controls ($^{*}P<0.05$).

CCR5, also were determined (Fig. 3). ADX caused significant increases in the mRNA expression of CCR2 (11-fold; $P<0.05$) and CCR5 (2.5-fold; $P<0.05$) receptors, respectively (Fig. 3B and C). CORT replacement reversed the effects of ADX on CCR2 mRNA (Fig. 3B) expression but did not significantly alter CCR5 mRNA expression (Fig. 3C). These findings suggest that CCR2 mRNA, like CCL2 mRNA is under strong negative regulation by endogenous CORT. An increase in the mRNA for the microglial marker, F4/80, also was observed after ADX and this effect was suppressed with CORT replacement (Fig. 3D), suggesting that CORT may be modulating microglial activation.

Microglial activation and the elaboration of microglia-associated cytokines and chemokines have been linked to various forms of neurological and neurodegenerative disorders. To confirm that damage of the hippocampus was associated with microglial activation, we analyzed the expression of mRNA for microglial markers and microglia-derived factors following the administration of the known hippocampal neurotoxicant, TMT. Administration of TMT to intact rats resulted in increased mRNA expression of the microglial markers, CD11b (564%; $P<0.05$; Fig. 4) and F4/80 (300%; $P<0.05$; Fig. 4), as well as, microglia-derived factors, IL-1α (380%; $P<0.05$; Fig. 4) and CCL2 (3500%; $P<0.05$; Fig. 5) in the hippocampus (Figs. 4 and 5). These findings indicate that damage to the hippocampus by TMT induces microglial activation and initiates the elaboration of microglia-derived cytotoxic cytokines and chemokines.

Trauma and chemically induced damage to the hippocampus are known to increase CCL2 mRNA, an effect often linked to microglial expression of this chemokine [3,6,9]. Various features of injury-related microglial activation are known to be suppressed by glucocorticoids [4]; therefore, we determined whether CORT would suppress the induction of CCL2 mRNA caused by TMT (Fig. 5). Administration of TMT to the intact rat resulted in the expected damage-induced astrogliosis, as assessed by increases in GFAP protein, 5–14 days post-dosing (data not shown), findings that are in agreement with our previous observations [1,6,12]. Moreover, our earlier finding of

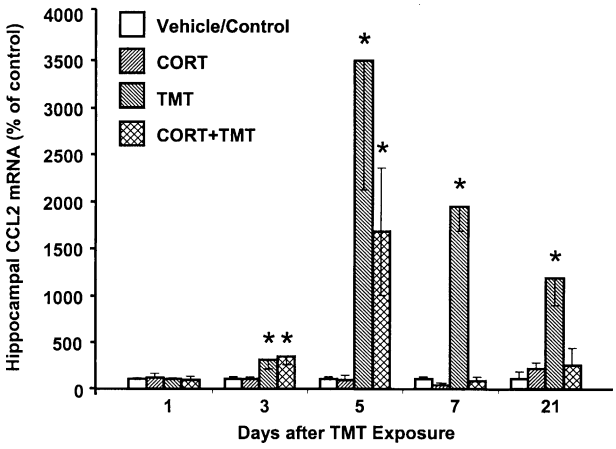

Fig. 5. TMT-induced expression of CCL2 mRNA in the hippocampus was suppressed by an immunosuppressive regimen of CORT. Rats were maintained on CORT (200 μg/ml) in drinking water commencing 1 week prior to administration of TMT (8 mg/kg, *i.p.*) and continued until sacrifice at different time points (1–21 days). Hippocampal mRNA expression of CCL2 was analyzed by real-time PCR. Values represent the mean ± S.E.M. for five independent observations and are expressed as percent of vehicle-treated controls. Significantly different from corresponding control ($^{*}P < 0.05$).

enhanced expression of CCL2 in association with TMT-induced hippocampal injury also was confirmed (Fig. 5). CCL2 mRNA was elevated in a time-dependent manner (Fig. 5) beginning at post-dosing day 3 (311%; $P < 0.05$), reaching peak expression at day 5 (3490%; $P < 0.05$) and persisting through post-dosing day 21 (1171%; $P < 0.05$). CORT treatment suppressed, but did not completely abolish, the time-dependent induction of CCL2 mRNA in hippocampus due to TMT (Fig. 5). In agreement with previous observations [12], TMT-induced astrogliosis was not affected by CORT in drinking water (data not shown).

Our findings demonstrate that manipulation of the glucocorticoid, CORT, can affect the expression of the chemokine, CCL2 and its receptor, CCR2 in the rat hippocampus. While the thymus-involuting CORT regimen used was not sufficient to regulate the expression of CCL2 in the intact rat, large increases in the expression of CCL2 were observed in ADX rats and these increases were suppressed following replacement therapy with physiological levels of CORT. These data indicate that CCL2 is under physiological regulation by CORT. Expression of the CCL2 receptor, CCR2, but not CCR5, was maintained under the same regulatory control of endogenous CORT. Potentially, this latter effect could be a result of the direct action of CORT on CCR2 or a secondary effect of CORT on CCR2 resulting from regulation of CCL2. Regardless of the exact underlying influences of CORT on CCL2 and CCR2, our findings are suggestive of a regulation of CCL2 signaling in hippocampus by physiological levels of CORT.

Neural damage, regardless of the origin of the insult, is a major stimulus for the activation of microglia and the attendant expression of microglial-associated cytokines and chemokines (e.g. see [17]). Previously, we have shown that enhanced expression of CCL2 is associated with OX-6 positive microglia, which are activated in response to hippocampal damage resulting from administration of the neurotoxic organometal, TMT [6]. When we used TMT in the present study to damage the hippocampus, we confirmed the expected increase in CCL2 and now we also have shown that the damage-associated expression of CCL2 can be suppressed with CORT. These data indicate that injury-induced expression of CCL2 in the hippocampus, an effect known to be associated with the activation of microglia, can be attenuated by prior and concurrent CORT therapy. This effect of CORT apparently is unrelated to injury-induced activation of astroglia, since the expression of the astrocyte marker GFAP was not affected, findings in agreement with our earlier observations [12]. The suppression of CCL2 and its receptor, CCR2, observed after CORT in the present study could be taken as evidence of suppression of microglial activation, given the association of CCL2 with activated microglia. Indeed, the enhanced expression of mRNA for the microglia marker, F4/80, and its suppression by CORT replacement therapy is consistent with this notion. F4/80, a 160 kDa cell surface glycoprotein is predominantly expressed by murine macrophages. Although no definitive function has been ascribed to F4/80, their expression on microglial cell surface is suggestive of a role in cell adhesion, phagocytosis, cellular immunity and perhaps signal transduction [7,16]. While it is tempting to infer that inhibition of microglial activation, as evidenced by decreased expression of CCL2 and other associated microglial-derived chemokines and cytokines, may serve to modulate the neuronal damage caused by TMT, such speculation is not necessarily warranted. For example, we recently found that suppression of multiple markers of microglial activation (including CCL2 and F4/80) by the tetracycline antibiotic, minocycline, does not affect the loss of dopaminergic nerve terminals caused by exposure to the dopaminergic neurotoxin, MPTP. Mice deficient in CCL2 also showed dopaminergic neurotoxicity due to MPTP that was indistinguishable from that observed in wild type mice [15]. Moreover, as with the effects of TMT in the present model, the astroglial response to MPTP was not modified in the face of the apparent suppression of microglial activation. Taken together, such observations suggest that injury-related expression of a number of microglial-associated chemokines and cytokines may subserve a role unrelated to the degree of damage caused by neurotoxic exposures affecting different neuronal targets; the broader implications of such findings remain unclear.

In summary, we have shown that CCL2 and its receptor CCR2 are under physiological regulation by CORT and that injury-induced expression of CCL2 can be suppressed by CORT, likely without affecting the degree of hippocampal damage caused by the neurotoxic exposure.

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