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Original experimental

Effect of intrathecal glucocorticoids on the central glucocorticoid receptor in a rat nerve ligation model

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HIGHLIGHTS

- The efficacy of neuraxial glucocorticoids for neuropathic pain is subject to debate.
- Glucocorticoids act mainly through their receptor (GR).
- Spinal nerve ligation increases spinal GR protein levels but not GR mRNA levels.
- After intrathecal glucocorticoid treatment only spinal GR mRNA decreases.
- This does not result in decreased ligation-induced mechanical hypersensitivity.

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ABSTRACT

Background and aims: Despite widespread use, the efficacy of neuraxial glucocorticoids for neuropathic pain is subject to debate. Since most glucocorticoid actions are mediated through its receptor, we explored the effects of intrathecal methylprednisolone acetate (MPA) on total glucocorticoid receptor (tGR) levels and activation of the glucocorticoid receptor (phosphorylated state = pGR) within the spinal dorsal horn (SDH) and dorsal root ganglion (DRG) in a spinal nerve ligation (SNL) model in rats.
Methods: Rats received unilateral ligation of the L5/L6 spinal nerves and were treated with two intrathecal doses of either 400 μg MPA or 0.9% saline with a 72-h interval. Plantar tactile thresholds were measured over time. Seven days after drug treatment, DRG and SDH were harvested to assess tGR and pGR levels using immunohistochemistry and qPCR.
Results: Allodynia, defined by lowered tactile withdrawal thresholds after SNL, was unaltered by intrathecal MPA. In saline controls, mRNA levels of *tGR* did not change after SNL in the DRGs or SDH. tGR and pGR protein levels in the SDH however, significantly increased on the ipsilateral side of SNL compared to the contralateral side and to naïve tissue. When treating rats with MPA, *tGR* mRNA levels were significantly reduced in the SDH compared to saline controls. tGR and pGR protein levels, however were not significantly lower compared to saline controls.
Conclusions: In intrathecal MPA treated rats, *tGR* mRNA levels decreased after SNL. However this did not result in lower tGR and pGR protein levels compared to saline controls, and did not decrease ligation-induced mechanical hypersensitivity.
Implications: Intrathecal MPA treatment after SNL did not result in lower tGR and pGR levels within the SDH and DRG compared to saline controls. In present study we did not differentiate between the various isoforms of the GR which might clarify this finding.

1. Introduction

As glucocorticoids act upon a variety of crucial targets in pain pathways [1], they should be potent long acting analgesic agents. However, despite widespread use of neuraxial glucocorticoids in pain medicine, their efficacy is subject to debate. There is consensus

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only on a short lasting analgesic effect in low back pain patients [2], but not in sustained pain states or for use in neuropathic pain syndromes. Preclinical and clinical results, in fact show varying effects of glucocorticoids from analgesic to hyperalgesic effects [3–10].

Our research group conducted a randomized controlled clinical trial in which we encountered disappointing analgesic effects with intrathecal methylprednisolone acetate (MPA) in patients suffering from postherpetic neuralgia [6]. Four intrathecal injections with MPA with 7-day intervals were administered in patients with intractable neuropathic pain. Patients treated with intrathecal MPA reported increased pain and with statistical evidence of futility, the trial was ended early. Our results were in sharp contrast with results of an earlier trial published in the *New England Journal of Medicine* with a similar drug and dosing regime, showing pain reduction in 92% of patients in the intrathecal MPA treated group [11]. Since we did not understand the differences in results between the two trials, we decided to conduct a preclinical study using a similar MPA formulation. In that study we researched the effect of intrathecal MPA in three established rodent pain models; carrageenan, formalin or spinal nerve ligation (SNL) model [12]. No acute analgesic effects were observed with intrathecal MPA in any of the three models. One of the questions that rose was if the glucocorticoid receptor is involved in the absence of the analgesic effects of intrathecal glucocorticoids.

On a cellular level, glucocorticoids mediate their actions primarily by binding to the glucocorticoid receptor (GR). The GR also known as NR3C1, is a ligand-driven transcription factor. Upon binding with a glucocorticoid, GR phosphorylates into an active form (pGR) and translocates to the nucleus where it affects expression of specific sets of genes by transcriptionally activating or repressing them [13]. In addition, glucocorticoids may evoke fast non-genomic neuronal responses by binding to membrane-bound or cytosolic GR or by effects not mediated by a receptor [13].

It is unclear exactly how glucocorticoids would act to regulate or modify pain signalling. After nerve injury, plasma cortisol levels and GR expression in the spinal cord are increased, indicating an elevated glucocorticoid activity [3,10,14]. Exogenous glucocorticoids may influence the endogenous increased plasma cortisol and GR expression in the spinal cord in several ways. They may increase GR binding and activity and stimulate its downstream actions, and down regulate endogenous cortisol levels and GR expression via a negative feedback mechanism. It is not known what the net effect of exogenous glucocorticoids on spinal GR levels in an acute pain state is. Therefore we examined if an intrathecal administered glucocorticoid, methylprednisolone acetate (MPA), alters (i) pain-like behaviour and (ii) total (tGR) and activated (pGR) glucocorticoid receptor levels within the spinal dorsal horn (SDH) and dorsal root ganglion (DRG) in a SNL model in rats.

2. Materials and methods

The protocol of the present study has been approved by the AAALAC accredited (International Association for Assessment and Accreditation of Laboratory Animal Care) Institutional Animal Care and Use Committee (IACUC) of the University of California, San Diego, USA.

2.1. Animals

Male Harlan Sprague-Dawley rats, 80–100 g (Indianapolis, IN, USA), were maintained 2 per cage in standard cages at room temperature on a 12:12 h light/dark cycle with free access to food and water. After arrival at the housing facility, they were allowed at least 2–3 days of acclimation before use. Experiments have been carried out during light cycle.

2.2. Spinal nerve ligation (SNL) model

Spinal nerve injury was induced by the procedure described by Kim and Chung [15]. Briefly, the left L5 and L6 lumbar spinal nerves were exposed in isoflurane 2.4%/oxygen-anesthetized rats and tightly ligated with 6.0 silk suture at a point distal to their DRGs and proximal to their conjunction to form the sciatic nerve. Rats were given post-operative subcutaneous fluids including analgesics (lactated Ringers + 5 mg/kg Carprofen) and then housed 2 per cage for post-operative recovery. Withdrawal thresholds were obtained at 0, 1, 3, 7, 18, 21 and 25 days after SNL for all rats.

2.3. Behavioural measurements

All behavioural measurements were made by observer (MR) blinded to the treatment groups and were conducted at fixed times (9:00 a.m. to 5:00 p.m.). The thresholds for mechanical allodynia were measured with a series of calibrated von Frey filaments (Stoelting, Wood Dale, IL, USA), ranging from 3.16 to 5.18 (0.41–15.0 g). The animals were acclimated for 45 min in the test chamber with mesh floors and von Frey filaments were applied perpendicularly against the plantar surface of the paw. The "up–down" method of Dixon as described by Chaplan [16] was used to determine the value at which paw withdrawal occurred 50% of the time, interpreted as the mechanical threshold.

2.4. Intrathecal catheter implantation and drug administration

On postoperative day 13 after SNL, when all rats were weighing more than 200 g, intrathecal catheters were implanted for drug injections. Rats were surgically implanted with intrathecal catheters under isoflurane 2.4%/oxygen inhalation anaesthesia as described previously by Yaksh and Rudy [17]. The catheter tip was located at the lumbar level of the rat spinal cord. Intrathecal catheters were externalized for injection. Rats were given post-operative subcutaneous fluids including analgesics (lactated Ringers + 5 mg/kg Carprofen) and then housed individually for post-operative recovery. Following implantation, catheters were flushed with saline and rats were monitored daily for viability, allowing 5 days of recovery before testing. Animals showing any evidence of motor dysfunction or distress after catheter placement were immediately euthanized using a carbon dioxide chamber.

On postoperative day 18 after SNL, rats were randomized to either the methylprednisolone acetate (MPA) group or the saline control group. Before administration, the presence of preservatives in the MPA preparation (depo-medrol® from Pfizer) was minimized as described in more detail before using saline as a vehicle [18]. The MPA group received 400 μg (10 μl) of the suspension followed by 10 μl 0.9% saline flush through the intrathecal catheter. In the saline group a total of 20 μl of 0.9% saline was injected.

Intrathecal injections were given twice with a 3-day interval, on postoperative days 18 and 21. This dosing interval was chosen based on pharmacokinetic data from our previous study showing that after intrathecal MPA administration, MP plasma levels went below the level of detection after 72 h [12]. We decided to expose rats to two periods of high levels of MPA before sacrifice, since the transcriptional activation or repression of genes by glucocorticoids can take 24–48 h [13].

2.5. Tissue collection

On postoperative day 25 after SNL, 7 days after the start of intrathecal drug treatment, spinal cord and dorsal root ganglia were collected from all rats and processed for either immunohistochemistry or quantitative real time PCR (qPCR). For immunohistochemistry, tissues were collected from rats subjected