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Spinal injection of IL-2 or IL-15 alters mechanical and thermal withdrawal thresholds in rats

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ABSTRACT

IL-2 and IL-15 were tested for effects on responses to mechanical or thermal stimuli when spinally administered to male Sprague–Dawley rats with surgically implanted intrathecal catheters. Restricted doses of both IL-2 and IL-15 produced increased responsiveness to mechanical stimulation of the hindpaws. This effect lasted up to 48 h. IL-2 had biphasic effects on thermal responses whereas IL-15 produced thermal hypalgesia alone. These effects dissipated within 24 h. These results suggest that IL-2 and IL-15 may participate in the generation of hyperalgesia in some pain conditions.

Cytokines are widely recognized as important mediators of many nociceptive processes [40,10]. For example, interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNFα) play key roles in the development and maintenance of chronic inflammatory diseases such as rheumatoid arthritis. Intradermal, intrathecal, or intraperitoneal injection of any of these alone or in combination cause mechanical hyperalgesia and increase various discharge properties of peripheral and dorsal horn neurons [29,13,27,30,22].

Less clear data are available on IL-2 and IL-15 in pain processes. IL-2 and IL-15 are functionally very similar pro-inflammatory cytokines that both bind and activate shared β and γ receptor subunits and similarly, though not identically, stimulate the proliferation of peripheral blood T, B and NK cells [38]. IL-2 and IL-15 and their receptors are also found in numerous nervous system structures including dorsal root ganglion, spinal cord, hippocampus, neostriatum, and hypothalamus [15]. T_H1 cells are considered the main source of IL-2 in the periphery, though B cells also produce IL-2 and IL-2 has been detected in primary cultures of neural tissue [17]. The main sources for IL-15 include epithelial cells, monocytes, fibroblasts, dendritic cells and activated T cells [38]. Through their immunomodulatory effects both IL-2 and IL-15 are implicated in the pathogenesis, albeit perhaps indirectly, of numerous painful and inflammatory conditions that involve clonal expansion of T and B cell subsets or auto-reactive cytotoxic T cells and antibodies. Examples of these conditions might include multiple sclerosis, human T lymphotropic virus type I-associated myelopathy/tropical spastic paraparesis, rheumatoid arthritis, sarcoiditis, ulcerative colitis, and inflammatory bowel disease [11,21,3].

Nevertheless, the direct effects of these cytokines on pain processes have either not been studied or been mixed. Intraplantar injection of recombinant IL-2 produced antinociception that was related to the binding of IL-2 to opioid receptors [32]. Similarly, the intrathecal administration of IL-2 and IL-2 gene transfection ameliorated thermal hyperalgesia in rats with chronic constriction injury of the sciatic nerve [42]. Contrary to these reports, the intravenous administration of IL-2 in humans for the treatment of various malignancies such as renal carcinoma, melanoma and neuroblastoma [11] have been associated with severe painful neuropathy refractory to analgesic medications, muscle aches, enhanced tumor-induced bone pain, and persistent localized pain at the site of the cytokine injection [28].

Amidst this controversy, IL-15 has been found in synovial fluid of patients with rheumatoid arthritis [20] and was found to be increased in dorsal root ganglion and the spinal dorsal horn of rats with vinca alkaloid (vincristine)-induced neuropathic pain [7]. Similarly, the expression of IL-2 receptor was increased in the DRG of rats with vincristine-induced chemoneuropathy [7]. IL-15 produced hyperalgesia when given intradermally in mice [35], but otherwise has not been tested for effects on pain processing. Hence, the goal of this study was to investigate the effects of intrathecal injection of IL-15 and to re-investigate the effects of intrathecal IL-2 on pain processing in rats.

A total of 58 male Sprague–Dawley rats (Harlan, Houston) weighing 350–400 g were used. Rats were individually housed at a room temperature of 22°. They were fed ad libitum and kept in a 12-h dark–light cycle. Behavioral experiments were conducted in a quiet room during the morning light period (9 a.m. to 12 noon). The

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investigator was blinded to the treatment administered during all of the behavioral tests. All experiments were conducted with Institutional IACUC review and approval and were in accord with the NIH guidelines to minimize pain or discomfort and the numbers of animals used.

A PE-10 catheter pre-filled with 7–8 μL of artificial cerebrospinal fluid was placed in the intrathecal space overlying the L1 spinal segment using previously described techniques [41,14]. Briefly, rats were anesthetized with 50 mg/kg of pentobarbital. Supplemental oxygen was provided during surgery. The posterior aspect of the neck was shaved, cleansed, incised and the atlanto-occipital membrane was exposed. The membrane was carefully opened, and the catheter advanced to the lumbar enlargement. Animals were allowed to recover for a minimum period of 5 days. No animals in this series showed any signs of motor impairment as a consequence of the catheter placement. At seven to 10 days after surgery, the animals received a single i.t. injection of 10 μL 1% lidocaine to induce a transient spinal anesthesia and thus verify proper catheter position. This was evidenced by flaccid hindlimbs and a lack of responses to toe pinch. At the end of all experiments the animals were sacrificed by pentobarbital overdose (300 mg, i.p.) and a laminectomy was performed. Dye was then injected via the catheter to visualize the placement of the tip. All animals in this series showed both hindlimb paralysis with spinal injection of lidocaine and dye spread over the lumbar enlargement during the post-mortem visualization and so were included for statistical analysis.

Behavioral testing began 10–14 days after surgery. Rats were previously acclimated to both the mechanical and thermal testing chambers by placing them into these chambers and allowing them to remain undisturbed for 15–30 min on three consecutive days between recovery from the surgery and the initiation of any test procedures. Assessment of mechanical and thermal withdrawal data was pseudorandomized for each rat on each testing day. Rats were allowed to return to their home cage for a 1 h rest between the first (mechanical or thermal) and the second assays on each day.

The rats were loosely restrained under clear acrylic boxes, placed on a wire mesh, and allowed to acclimate on each experimental day for at least 15 min. Von Frey filaments with bending forces of 0.12, 0.53, 1.05, 4.41, 8.86, 16.4, and 42 g were presented from below in pseudorandom order and held in bent position for 1–2 s on the mid-plantar surface of the hind paw. Each foot was stimulated with the same filament five times with an interstimulus interval of 3–5 min. The total number of paw withdrawals for each filament was recorded. A response was defined as brisk foot withdrawal in response to the mechanical stimulus [7].

Thermal nociceptive thresholds were assessed using radiant heat [16]. The rats were loosely restrained under arcylic boxes placed on a glass surface for a conditioning period of at least 15 min. The heat source was positioned beneath the mid-plantar surface of the hind paw. Withdrawal latency was defined as the period of time from the beginning of the thermal stimulation to the brisk withdrawal of the hind paw. To avoid tissue damage, a cutoff time of 24 s was set. Thermal stimulation was applied three times to each hind paw in pseudorandomized order at an interstimulus interval of 3–5 min [7].

IL-2 (recombinant rat) and IL-15 (recombinant human) were purchased from Biosource International (Carmarillo, CA, Catalog Nos. PRC0024 and PHC0154, carrier-free lyophilized protein). Both cytokines were injected in a volume of 10 μL followed by the administration of 10 μL of artificial cerebrospinal fluid (ACSF). The dose ranges were based on precedent studies of IL-2 effects in the peripheral and central nervous system [32,44,31] and based on measurements of cytokine responses in neural tissue induced in models of experimental neuropathy. Two doses of IL-2 were used in reference to the earlier studies: 500 and 2000 U. These are referred to here as the IL-$2_{500\,U}$ and IL-$2_{2000\,U}$ groups. Given the limited knowledge of IL-15 effects in the CNS, three doses of this cytokine were used: 500, 1000, and 10,000 U. These are referred to here as the IL-$15_{500\,U}$, IL-$15_{1000\,U}$, and the IL-$15_{10,000\,U}$ groups. Denatured IL-2 (2000 U) and IL-15 (500 and 1000 U) were injected to verify specific activity of the protein and to exclude non-specific effects of foreign protein. These groups are referred to as the $IL2_{Denatured}$ and the $IL15_{Denatured}$ groups, respectively. The composition of the ACSF was standard based on previous behavioral and physiological studies in the laboratory (e.g. [14]) and contained the following (in mM): NaCl 120; KCl 3; $NaHCO_3$ 25; $CaCl_2$ 2.5; $MgCl_2$ 0.5; glucose 12 (pH 7.4).

The percent mechanical withdrawal threshold and the thermal withdrawal latencies are expressed as mean ± standard error. To evaluate statistical significance between treatment groups in mechanical withdrawal threshold the Kruskall–Wallis nonparametric analysis was used. The effects of each agent over time were analyzed using ANOVA. Post hoc evaluations in the latter analysis were performed using Tukey's test. Significant difference was considered at $p < 0.05$.

The spinal administration of denatured IL-2/IL-15 in ACSF did not produce any change in response to mechanical stimulation of the hindpaws. Fig. 1 shows the mean responses for each treatment group to the highest threshold 42 g von Frey filament in the test series over time in comparison with the data obtained appropriate control groups (A and C). This strength filament produced constant responses at approximately 15% of all trials throughout the experiment for the control group. The withdrawal responses in the control rats to other filaments were similarly stable albeit at lesser rates of response. The range of filaments where responses were obtained also remained constant down to the 4.41 g filament. The mean response at this lowest strength filament was approximately 6% at all trials (mean 5.8 ± 2% for the control group at baseline). Intrathecal injection of 500 U IL-2 had no effects on mechanical withdrawal responses (Fig. 1). These animals also showed overall responses over the same range of filaments as the control rats (4.41–42.0 g filaments), and the rates of responses to all filaments were comparable to the control rats.

In contrast, intrathecal IL-2 at a dose of 2000 U induced mechanical hyper-responsiveness. This is evidenced in Fig. 1A by an increase in the percentage of hind paw withdrawal in response to the 42 g force. The baseline responses were comparable in both groups; however, by 1 h after injection the response of the IL-$2_{2000\,U}$ group had increased to 39.1 ± 8.5% whereas the control rats maintained stable responses at 11.7 ± 3.5% ($p < 0.05$ vs. matching time IL-$2_{Denatured}$, and IL-$2_{2000\,U}$ baseline). The responses to mechanical stimulation remained elevated when measured at 3 (IL-$2_{Denatured}$: 12.7 ± 3.1% vs. IL-$2_{2000\,U}$: 35.5 ± 5.5%; $p < 0.05$ matching time $IL2_{Denatured}$, and IL-$2_{2000\,U}$ baseline), 6 (controls: 12.7 ± 3.5% vs. IL-$2_{2000\,U}$ 45.5 ± 8.5%, $p < 0.05$ matching time $IL2_{Denatured}$, and IL-$2_{2000\,U}$ baseline) and 24 h (IL-$2_{Denatured}$: 15.45 ± 5.36% vs. IL-2: 45.45 ± 8.04%; $p < 0.05$ matching time IL-$2_{Denatured}$, and IL-$2_{2000\,U}$ baseline) after cytokine injection. Responses were similarly elevated to the other filaments in the test series that were effective in evoking responses in the baseline tests (4.41–42 g). These data are shown in Fig. 1B. The filaments not effective at evoking responses in the baseline (0.12–1.05 g filaments) remained ineffective following IL-$2_{2000\,U}$ except for that the 1.1 g filament evoked 3.6 ± 1.3% responses at 1 h following cytokine injection (not shown). Mechanical responsiveness was found to be returning toward normal by 48 h after cytokine injection and showed full recovery by 1 week.

Spinal administration of 500 and 10,000 U IL-15 produced no change in the responses to mechanical stimulation. However, the intermediate dose of 1000 U IL-15 induced mechanical hyperalgesia in a pattern very similar to that observed in the IL-$2_{2000\,U}$ group. The

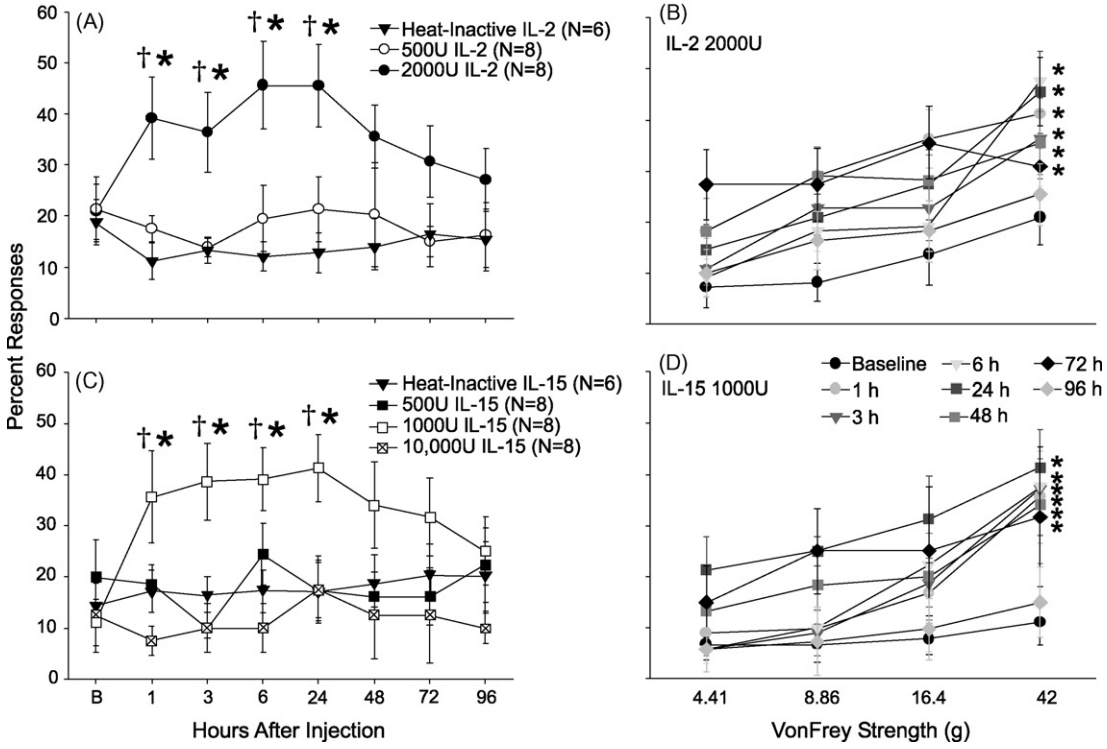

Fig. 1. Scatter line plots in (A) and (C) show the response rates of rats to a 42 g von Frey filament at baseline (B) and then at several time points following intrathecal (i.t.) injection of heat-inactivated protein (solid triangles, upper and lower panels), IL-2_{500U} (open circles, upper panel), IL-2_{2000U} (filled circles, upper panel), IL-15_{500U} (filled squares, lower panel), IL-15_{1000U} (open squares, lower panel) or IL-$15_{10,000U}$ (cross-hatched squares, lower panel). IL-2_{2000U} and IL-15_{1000U} both produced hyperresponsiveness to mechanical stimuli evidenced by the increased percent of positive withdrawal responses by 1 h following i.t. injection and this effect persisted for 24 h. Responses to mechanical stimuli remained elevated, though not significantly so, for up to 3 days and then slowly returned to baseline levels. Responses to all filaments for IL-2_{2000} and IL-15_{1000U}, the doses of each cytokine that produced mechanical hyperalgesia, are shown over time in (B) and (D). In (A) and (C), $^{*}p < 0.05$ vs. combined baseline values, $^{\dagger}p < 0.05$ vs. heat-inactivated protein control. In (B) and (D), $^{*}p < 0.05$ vs. matching baseline (AUC Comparison).

rate of response to the 42 g filament was increased to 35.6 ± 9.0% 1 h following cytokine injection ($p < 0.05$ matching time IL-$15_{Denatured}$, and IL-1_{5000U} baseline) and this effect persisted when measured at 3, 6, and 24 h. Similar increases in responses were observed in the other filaments effective at baseline (4.41–42 g) and shown in Fig. 1D. No responses developed in the filaments ineffective at baseline (0.12–1.05 g). Finally, like observed in the IL-2_{2000U} group, the responses to mechanical stimulation returned toward baseline at 48 h in the IL15_{1000U} group, and showed full recovery by 1 week.

Denatured IL-15/IL-2-ACSF did not modify paw withdrawal latency to radiant heat. As shown in Fig. 2, paw withdrawal occurred at 13.0 ± 1 s in the baseline assessment. Later assessments yielded values very close to the baseline ranging from a high of 14.3 ± 1.3 s at 6 h after injection to a low of 12.8 ± 1.0 s at the 48 h time point. Baseline paw withdrawal latencies for the remaining treatment groups also fell in this same range of times (Fig. 2).

Intrathecal administration of IL-2 produced two patterns of change in response to thermal stimulation. The higher dose of 2000 U IL-2 produced a transient thermal hyperalgesia present when measured at 1 and at 3 h after treatment. In contrast, intrathecal injection of 500 U IL-2 produced a modest increase in paw withdrawal latency when measured at 1 and 3 h after injection. This increase was significantly different by a within group comparison to baseline, but was not sufficiently large in magnitude to achieve statistical significance in comparison to the IL-$2_{Denatured}$ group. Both effects, the IL-2_{2000U} induced hyperalgesia and the IL-2_{500U} induced hypalgesia dissipated by 24 h following cytokine injection.

Intrathecal injection of IL-15 produced only a single pattern of change in response to radiant heat. Animals in the IL-15_{500U} and IL-15_{1000U} groups each showed increased withdrawal latencies to radiant heat at 1, 3 and 6 h following cytokine injection. This increase was significantly different from the within group baseline and the IL-$15_{Denatured}$ group for the IL-15_{1000U} group at each of these time points, but was only significantly different in the IL-15_{500U} from the within-group baseline at the 3 h time point. These effects had resolved by 24 h following cytokine injection and the responses to thermal stimulation remained in the normal range for the remainder of the experiment. No effect of intrathecal injection of 10,000 U of IL-15 on the responses to radiant heat were observed.

IL-2 has many well-documented effects on forebrain neurons [15]. IL-2 modulates the maturation, survival and connectivity of forebrain neurons [4]. In the adult brain, IL-2 penetrates the blood-brain barrier [37] and exerts modulatory effects on forebrain-mediated neuroendocrine and behavioral functions. Thus, IL-2 alters the physiology of neurons in the ventromedial, supraoptic and paraventricular nuclei of the hypothalamus [5] resulting in alterations in the secretion of multiple hormones including oxytocin [23] and especially pro-opiomelanocortin-derived hormones and peptides [6,18]. IL-2 produces profound psychological and cognitive impairments in both experimental animals and human patients [44,12,9]. These effects may be mediated by modulation of forebrain dopamine systems [25] and suppression of hippocampal long-term potentiation [34], that in turn may be mediated by direct partial inhibition by IL-2 of calcium, NMDA and kainite receptor-mediated currents in hypothalamic, hippocampal and tegmental neurons [31,43,26]. Prolonged systemic or intrathecal infusion of IL-2 in adults results in toxicity to oligodendrocytes, promotes demyelination and toxicity to neurons [15,4].

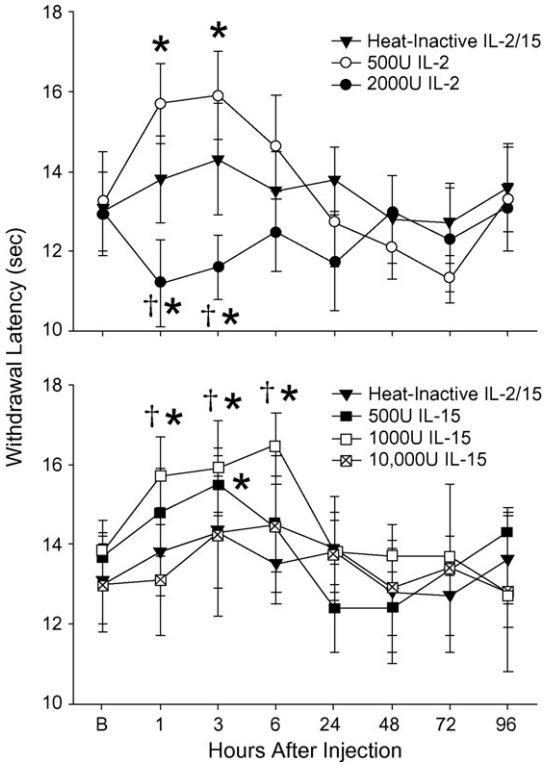

Fig. 2. The scatter line plots show the mean withdrawal latency to radiant heat at baseline (B) and then at several time points following intrathecal (i.t.) injection of heat-inactivated protein (solid triangles, upper and lower panels), IL-2_{500U} (open circles, upper panel), IL-2_{2000U} (filled circles, upper panel), IL-15_{500U} (filled squares, lower panel), IL-15_{1000U} (open squares, lower panel) or IL-$15_{10,000U}$ (cross-hatched squares, lower panel). IL-2_{2000U} produced hyper-responsiveness to thermal stimuli evidenced by the decreased latency to paw withdrawal to radiant heat by 1 h following i.t. injection and this effect persisted for 3 h and then returned to baseline. In contrast, IL-2_{500U} and IL-15_{500U} and IL-15_{1000U} produced thermal hypalgesia (analgesia) that similarly was evidenced within 1 h by an elevation in paw withdrawal latency to radiant heat (3 h for IL-15_{500U}) and this effect persisted between 3 and 6 h. All responses returned to the baseline level by 24 h following i.t. injection. $^{*}p < 0.05$ vs. combined baseline values, $^{\dagger}p < 0.05$ vs. heat-inactivated protein control.

There is less cumulative history, and as well less agreement, concerning the role of IL-2 in pain processing. One set of evidence indicates that increased levels of IL-2 in and around sensory nerve endings or in the central nervous system promote nociceptive behaviors. Systemic administration of IL-2 in cancer patients induces pain at the site of injection, bone pain, and painful peripheral neuropathy [2,1,36]. Experimental studies in animals replicate many of these clinical observations. Intraplantar injections of IL-2 in rats produced mechanical hyperalgesia that was inhibited by the previous administration of anti-IL-2 antibody [24]. Similarly, intraarticular injections of IL-2 into the knee joints of rats produced mechanical hyperalgesia with a maximum peak effect at 1 h after the injection that was prevented and reversed by the administration of a bradykinin receptor 1 antagonist [8]. These effects appear to be mediated by depolarization by IL-2 of discrete subpopulations of polymodal nociceptors [19].

Nevertheless, in another series of papers IL-2 induced thermal antinociception in rats that was naloxone-reversible, suggesting an interaction with opioid receptors (e.g. [33]). As noted above, these findings have support in other CNS studies wherein it was observed that IL-2 induces the release of opioid peptides [18] and IL-2 hyperpolarized DRG neurons by interactions at μ opioid receptors [32].

The results shown here help resolve the apparent conflict in the role of IL-2 in nociceptive processing in that our findings confirm both lines of study. Intrathecal injection of 500 U of rat recombinant IL-2 produced thermal antinociception, whereas higher doses produced thermal and mechanical hyperalgesia. The discovery of this biphasic response to the species-specific protein, typical of many effects of cytokines [21], suggests that perhaps one set of responses are mediated by interactions of the protein with high-affinity receptors, whereas other responses, particularly those at higher doses and with cross-species proteins, may be mediated at low-affinity receptors. Thus, the analgesic dose required in previous studies [33] was significantly higher than the dose used in the present study possibly due to the use of cross-species protein. Similarly, the time course to the analgesic effects observed by this group was very short, on the order of minutes [33], whereas in this report the effects lasted several hours. These discrepancies may be accounted for by the fact that we used rat-IL-2 and whereas this other group used human recombinant protein. Interestingly our findings with recombinant human IL-15 are in accord with all the findings of this group with human IL-2 except in duration of effects. Presumably, the high-affinity effects of each cytokine would be mediated by the private α receptor subunits, whereas the lower-affinity sites would potentially be mediated by the shared βγ subunits [39].

In conclusion, the spinal administration of IL-2 and IL-15 induces transient changes in the responses of rats to mechanical and thermal stimuli. These results suggest that these cytokines should be considered as additional members of CNS-active immune-derived signal molecules that likely have important roles in inflammatory and neuropathic pain conditions.

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