Published in final edited form as:

Pain. 2010 April ; 149(1): 107–116. doi:10.1016/j.pain.2010.01.017.

Cellular Basis for Opioid Potentiation in the Rostral Ventromedial Medulla of Rats with Persistent Inflammatory Nociception

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Abstract

Direct inhibition of pain facilitatory neurons in the rostral ventromedial medulla (RVM) is one mechanism by which *mu* opioid receptor (MOPr) agonists are proposed to produce antinociception. The antinociceptive and anti-hyperalgesic effects of the MOPr agonist DAMGO are enhanced after intraplantar injection of complete Freund's adjuvant (CFA). This study therefore examined whether CFA treatment similarly enhanced the ability of DAMGO to induce outward currents in spinally projecting RVM neurons. It further examined whether the electrophysiological properties of RVM neurons are altered by CFA treatment. Whole-cell patch clamp recordings were made from three types of serotonergic as well as non-serotonergic spinally projecting RVM neurons obtained from control rats and rats four hours or four days after CFA. Persistent, but not acute inflammatory nociception increased the percentage of Type 2 non-serotonergic neurons that responded to DAMGO from 17% to 57% and the percentage of Type 3 serotonergic neurons that responded to DAMGO from 5% to 55%. These same two populations of RVM neurons exhibited significant differences in their passive membrane properties or spontaneous discharge rate. The outward currents produced by the GABA_B receptor agonist baclofen were not enhanced, suggesting that the enhancement does not reflect global changes in levels of G_{i/o} or activity of G protein regulated inwardly rectifying potassium channels. These results provide a cellular basis for the enhanced anti-hyperalgesic and antinociceptive effects of MOPr agonists under conditions of persistent inflammatory nociception. These results also provide intriguing, albeit indirect, evidence for two different populations of pain facilitatory neurons in the RVM.

INTRODUCTION

Neurons in the nucleus raphe magnus and the adjacent nucleus reticularis gigantocellularis pars α , collectively termed the rostral ventromedial medulla (RVM), give rise to bulbospinal pathways that can either facilitate or suppress sensory transmission in the dorsal horn. Peripheral injury leads to sustained time-dependent changes in the pharmacology and physiology of these neurons, and in the balance of activity in bulbospinal pain facilitatory

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and inhibitory pathways. These changes have largely been inferred from findings that peripheral inflammatory injury causes a time-dependent enhancement of the antinociceptive and anti-hyperalgesic effects of opioid [23,24,41], N-methyl-D-aspartate (NMDA) [18,45], α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) [17,18,45], or GABA_A [14] receptor agonists in the RVM. Extracellular recording methods have established that peripheral injury alters the activity of RVM neurons [3,16,27,39]. However, technical limitations preclude an analysis of how injury alters the properties of these neurons and hinder determination of their axonal projections. None of these studies have addressed which populations of neurons mediate injury-induced changes in the potency and efficacy of drugs in the RVM.

The first aim of the present study was to determine whether acute or persistent inflammatory nociception altered the passive membrane, action potential and discharge properties of any of the different types of spinally projecting RVM neurons. Whole-cell patch clamp recordings were made from different types of serotonergic and non-serotonergic spinally projecting RVM neurons [50] in brainstem slices from three different treatment groups: control (naïve or saline-treated), acute inflammatory injury, and persistent inflammatory injury produced by injection of complete Freund's adjuvant (CFA). The second aim was to determine which population(s) of neurons may mediate the enhanced antinociceptive and anti-hyperalgesic effects of the *mu* opioid receptor (MOPr) agonist, D-Ala²,NMePhe⁴, Gly⁵-ol]enkephalin (DAMGO) after inflammatory injury. *Mu* opioid receptor agonists are thought to act in the RVM by two mechanisms to produce antinociception. The first involves disinhibition of spinally projecting pain inhibitory neurons [6]. The second proposal, for which there is less evidence, involves direct inhibition of bulbospinal pain facilitatory neurons [34]. This study therefore compared the ability of DAMGO to produce outward currents in the different populations of serotonergic and non-serotonergic RVM neurons in control and CFA-treated rats. It further examined whether the enhancement of DAMGO's effects was secondary to changes in downstream signaling mechanisms, such as an increase in levels of G_{i/o} proteins or activity of G protein regulated potassium channels (Kir). This possibility was probed with baclofen, an agonist at the GABA_B receptor that couples to the same G_{i/o} protein and Kir channels as DAMGO [2,31,35]. The results provide intriguing, albeit indirect, evidence for two distinct populations of pain facilitatory neurons in the RVM. These results also provide further support for the idea that opioids may produce their anti-hyperalgesic or antinociceptive effects by direct inhibition of spinally projecting RVM neurons. Identification of robust phenotypic markers that can identify the functional nature of the different populations of RVM neurons can facilitate their study *in vitro* and *in vivo*.

METHODS AND MATERIALS

These experiments were approved by The University of Iowa Animal Care and Use Committee. They were conducted in accordance with the guidelines of the International Association for the Study of Pain and the National Resource Council Guide for the Care and Use of Laboratory Animals. Every effort was made to minimize the number of rats used, as well as their stress and discomfort.

Animals

Male Sprague Dawley rats 11- to 18-days of age (Charles River) were used. Limited neuronal viability and visibility restrict whole-cell patch clamping recordings in the RVM to rats $\leq$ 18 days of age. Thus, in considering these results, both similarities and differences in the bulbospinal pathways of neonates and adults must be recognized. Similarities include the fact that degenerating bulbospinal terminals are present in the lumbar spinal cord as early as PD4, and exhibit adult-like patterns of degeneration by PD15 [15]. Also, electrical stimulation of the dorsolateral funiculus inhibits C-fiber evoked responses of dorsal horn

neurons after PD10, with reasonably strong inhibition present at PD18 and adult strength of inhibition evident by PD21 [9]. Finally, as in the adult, electrical stimulation of the RVM at P21 can inhibit or facilitate the responses of deep dorsal horn neurons to noxious mechanical stimuli [22]. Differences include the recent finding that the percentage of deep dorsal horn neurons that exhibit facilitation is much larger at P21, that none exhibit the adult pattern in which facilitation transitions to inhibition as stimulus intensity increases, and the net effect of RVM stimulation up to P21 is facilitation of the EMG response to mechanical stimulation of the hindpaw [22].

Retrograde Labeling of Spinally Projecting Neurons in the RVM and Induction of Persistent Inflammation

After induction of anesthesia and a small laminectomy, a 0.5 mm³ pledget of Gelfoam® soaked in 0.2% DiI (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate in 100% DMSO; Molecular Probes, Eugene, OR) was introduced under the dura [50]. This method retrogradely labels serotonergic and non-serotonergic neurons in young rats [50] in proportions similar to those reported for the adult rat [20,43]. Figure 1 illustrates the distribution of DiI in two rats. This low concentration of DiI does not interfere with currents evoked by G_{i/o}-coupled receptor agonists [49].

While recovering from anesthesia, pups were injected in the plantar surface of each hindpaw with 40 µl of pH 7.4 saline or complete Freund's adjuvant (CFA; Calbiochem; San Diego, CA). The immune system of neonatal rats is not well developed [8]. Indeed, hindpaw inflammation was substantially diminished two days after the first injection and had subsided by four days. Therefore, two days later, a second injection of CFA was made to ensure a state of persistent inflammatory nociception. Controls received a second injection of 20 µl saline. Some pups, referred to as naïve animals, received no injection of saline. Saline-treated and naïve rats were grouped together as a single control group because the passive membrane and action potential properties of each type of neuron in saline-treated rats (18 serotonergic and 53 non-serotonergic neurons) did not differ from those recorded in naïve rats (54 serotonergic and 76 non-serotonergic neurons) (data not shown). A subset of pups was injected with 40 µl of CFA and killed four hrs later to examine the effects of acute inflammatory nociception. The ages of control and CFA-treated rats at the time of recording were comparable for each type of neuron with one exception (Supplemental Fig. 1A).

Slice Preparation and Recording Conditions

Three- to five-days after surgery, the pups were decapitated, the brain was rapidly removed and coronal slices of 160 µm thickness containing the RVM were cut as described [50]. Brainstem slices containing the locus coeruleus (LC) were also taken in some instances. After equilibration in oxygenated artificial cerebrospinal fluid for at least one hr at 34°C, slices were transferred to the recording chamber and continuously perfused with oxygenated artificial cerebrospinal fluid at 32-34 °C (4 ml.min⁻¹). Retrogradely labeled neurons were visualized by epifluorescent illumination with a rhodamine filter.

Whole cell voltage clamp recordings were made using glass recording pipettes of 3-6 MΩ resistance that contained (in mM): 140 K⁺ methane sulphonate, 10 HEPES, 2 MgCl₂, 0.6 EGTA, 2 MgATP, 0.25 Na₂GTP; pH 7.3, 280-290 mOsm. Biocytin (0.01% w/v) was included in the pipette to enable subsequent identification of the neurons. This concentration is 100-fold less than the concentration that alters the passive membrane properties of substantia gelatinosa neurons and diminishes the proportion of these neurons that are hyperpolarized by a MOPr agonist [5]. The holding potential was maintained at -60 mV with an Axopatch 200B amplifier (Axon Instruments, Union City, CA). Series resistance

was compensated by 70-80%. A seal resistance of $\geq 3 \text{ M}\Omega$ and access resistance of $< 25 \text{ M}\Omega$ were required. Liquid junction potentials of -14 mV were uncorrected.

Passive membrane properties were measured in voltage clamp mode at a holding potential of -60 mV . Resting membrane potentials and action potentials were recorded in current clamp mode, sampled at 10 kHz , and filtered with a 5 kHz low-pass filter. Interspike interval (ISI) was measured for a period of 30 s using the Minianalysis program (Synaptosoft, Decatur, GA) supplemented with visual verification. The coefficient of variation (CV_{ISI}) was calculated as the $\text{SD}/\text{mean ISI}$ [29]. A CV_{ISI} of 0 corresponds to no variation in firing rate, while a value > 1 would correspond to highly irregular or bursting activity. Details of these measurements appear elsewhere [48,50].

All RVM neurons recorded in this study were spinally projecting. Outward currents induced by bath application of the MOPr agonist DAMGO, the mixed DOPr/MOPr agonist $[\text{Met}^5]\text{enkephalin}$, or the GABA_B receptor agonist baclofen were recorded in voltage clamp gap-free mode (holding at -60 mV , sampled at 10 kHz , pClamp low pass filter 50 Hz). A neuron was considered opioid- or baclofen-responsive if the agonist induced an outward current $\geq 10 \text{ pA}$ that was reversed by subsequent application of $1 \text{ }\mu\text{M}$ naloxone or $30 \text{ }\mu\text{M}$ CGP35348, respectively. The responsiveness of RVM neurons to DAMGO did not differ between the ages of 11 and 18 days of age (Supplemental Fig. 1B).

Immunohistochemical Procedures and Classification of Neurons

After recording, slices were fixed by immersion for one hr in 4% paraformaldehyde- 0.1 M phosphate buffer. Slices were processed for tryptophan hydroxylase (TrPH) immunoreactivity using mouse anti-tryptophan hydroxylase (1:500; Oncogene OP71L; Cambridge, MA) or, in the case of LC neurons, for tyrosine hydroxylase using mouse anti-tyrosine hydroxylase (1:2000, Immunostar; Hudson, WI). The neuron from which the recording was made was identified by its biocytin content. Details of the immunohistochemical procedures are provided in Zhang et al. [49,50]. After confirming under low magnification that the neuron was in the RVM or the LC, the sections were examined with a Bio-Rad 1024 confocal microscope using sequential, single laser line scanning methods to obtain serial stacks for each fluorophore throughout the entire z-axis of the labeled neuron. Figure 2 illustrates representative locations of recorded neurons in the RVM.

Neurons were considered noradrenergic if they were immunoreactive for tyrosine hydroxylase and serotonergic if they were immunoreactive for TrPH. As was done for dorsal and median raphe neurons [1,28,33], we analyzed the passive membrane and action potential properties of RVM neurons that were immunoreactive for TrPH [50] and identified criteria (e.g. action potential half-width, membrane resistance) that were highly predictive of the serotonergic nature of a neuron. Using those criteria, zero of 13 Type 1 neurons (0%), one of 22 Type 2 neurons (4.5%), and two of 21 Type 3 neurons (9.5%) from saline-treated rats exhibited the electrophysiological characteristics of serotonergic neurons yet were not immunoreactive for TrPH. In four-day CFA-treated rats, only three of 40 Type 1 neurons (7.5%), one of 64 Type 2 neurons (1.5%), and two of 49 Type 3 neurons (4.1%) exhibited this discrepancy. Given the small percentages and the previous finding that dialysis of cellular contents can lead to a false negative with only an immunohistochemical approach [50], these few neurons were included as serotonergic for the purposes of analysis.

Drugs

($\pm$) Baclofen and naloxone hydrochloride were purchased from Research Biochemicals (Natick, MA). DAMGO and $[\text{Met}^5]\text{enkephalin}$ were purchased from Sigma (St. Louis, MO).

Tetrodotoxin (TTX) was purchased from Sigma or Alamone Laboratories (Jerusalem, Israel). All drugs were dissolved in distilled water, aliquoted, and stored at -20°C or -80°C until use. Drugs were applied by addition to the ACSF and delivered by pump with a BPS-8 channel valve control perfusion system (ALA Scientific Instruments; Westbury, NY). Equilibrium concentrations of drug were achieved in the recording chamber by 90 sec.

Statistical Analysis

The passive membrane and action potential properties of RVM neurons were compared by a two-way analysis of variance in which one factor was treatment (control vs CFA) and the other factor was cell type. Post-hoc comparisons were made by Newman Keul's test. A $P < 0.05$ was considered significant for this and all other analyses. Concentration-effect curves were fit by least squares linear regression. Where the data were quantal, as in the percentage of responsive neurons, the percentages were first converted to probits. The EC_{50} was defined as the concentration that produced a response (≥ 10 pA) in half the number of neurons that responded to a maximally effective concentration of drug; 95% confidence limits (95% CL) were determined using PharmTools Pro (McCary Group; Philadelphia, PA). In the case of outward currents, which were not quantal, the data were not transformed. Here, the EC_{50} was defined as the concentration that produced an outward current that was one-half the amplitude of the current produced the highest concentration of drug; 95% CL were determined using Fieller's theorem as applied by Finney [7]. The slopes and intercepts of the concentration-response curves of different treatment groups were compared by analysis of co-variance. Fisher's Exact Test was used to compare percentages of drug-responsive neurons among different treatment conditions. Where a 2×3 table was analyzed the Freeman Halton extension of the test was used [10, available at (<http://faculty.vassar.edu/lowry/VassarStats.html>)]. Where an overall significant difference was identified, the table was separated into 2×2 subtables for further analysis to identify the groups responsible for the difference.

RESULTS

Persistent, but not Acute Inflammatory Nociception Alters the Passive Membrane and Action Potential Properties of Two Types of Spinally Projecting RVM Neurons

As in naïve rats [50], three general types of spinally projecting RVM neurons could be discerned in saline- and CFA-treated rats. The passive membrane and action potential properties of these neurons in control and CFA-treated rats are presented in Tables 1 and 2. Figure 3 illustrates key differences in their discharge patterns. Type 1 serotonergic and non-serotonergic neurons discharged intermittently or in bursts separated by periods of quiescence. This pattern was reflected in their large CV_{ISI} values (Fig. 3A, D), variance in the ISI values (Fig. 3B, E), and low rate of spontaneous discharge (Fig. 3C, F). Type 2 serotonergic and non-serotonergic neurons were not spontaneously active. Type 3 serotonergic and non-serotonergic neurons fired repetitively and in a very regular manner as indicated by their very small CV_{ISI} values (Fig. 3A, D), decreased variance in ISI values (Fig. 3B, E) and higher rate of spontaneous discharge (Fig. 3C, F). Serotonergic Type 1 and 3 neurons discharged at a lower frequency than non-serotonergic neurons of the corresponding type ($P < 0.05$ and 0.01 , respectively; Fig. 3C, D) and had longer ISI's ($P < 0.05$ for both; Fig. 3C, F).

Persistent inflammatory nociception did not change the passive membrane and the action potential properties of non-serotonergic Type 1 or Type 3 neurons. It also did not alter the CV_{ISI} , ISI or spontaneous discharge of these neurons (Fig. 3A-C). However, Type 2 non-serotonergic neurons exhibited a significantly more negative resting membrane potential than in control rats (Table 1).

Persistent inflammatory nociception also did not affect the passive membrane and action potential properties of Type 1 serotonergic neurons (Table 2). However, the CV_{ISI} for this population was modestly decreased four days after CFA treatment, consistent with a slight decrease in the irregularity of the spontaneous discharge (Fig. 3D). The passive membrane and action potential properties of Type 2 serotonergic neurons were also unchanged four days after CFA (Table 2). In contrast, Type 3 serotonergic neurons from CFA-treated rats exhibited a significant decrease in capacitance (Table 2), as well as a significantly higher frequency of spontaneous discharge and a corresponding decrease in ISI (Fig. 3D-F).

Acute inflammatory nociception, modeled as four hrs after CFA treatment, did not alter the passive membrane, action potential and discharge properties of Type 1, 2 or 3 non-serotonergic neurons (data not shown). It also did not alter the passive membrane and action potential properties of serotonergic Type 1, 2 and 3 neurons (data not shown). However, far fewer Type 2 serotonergic neurons were encountered four hrs after CFA treatment, i.e. only two of 24 serotonergic neurons were identified as Type 2. Given that the half-life of TrpH in neurons is ~1.6 days [38,46], it is unlikely that levels of TrpH in these neurons decreased below those detectable by immunohistochemistry. A more likely explanation is that these neurons became spontaneously active, shifting to the Type 1 or Type 3 classification in the period immediately after injury.

DAMGO- and [Met⁵]enkephalin Produce Modest Currents in Spinally Projecting RVM Neurons in the Absence of Inflammatory Injury

As expected, DAMGO produced an outward current in serotonergic and non-serotonergic RVM neurons that was completely reversed by 1 μ M naloxone (Fig. 4A) and persisted in the presence of 1 μ M tetrodotoxin (see Fig. 7 of [50]). However, only 11 of 36 (30.6%) non-serotonergic and 9 of 31 (29.0%) serotonergic neurons responded to the highest concentrations of DAMGO (Figure 5A,B). Moreover, the amplitude of the current was only ~25 pA and not concentration-dependent (Fig. 5C,D). In contrast, DAMGO evoked large, concentration-dependent outward currents when applied to LC neurons in rats of the same age, and in some cases the same rats from which RVM slices were taken (0.3 μ M: 52 ± 9.7 pA in all 4 neurons; 1.0 μ M: 98.7 ± 14.0 pA in 6 of 7 neurons; 3.0 μ M: 135.7 ± 9.7 pA in all 6 neurons). [Met⁵]enkephalin (10 μ M) similarly produced modest outward currents (Fig. 4B) in RVM neurons (34 ± 12 pA in 3 of 6 Type 1, 43 ± 12 pA in 3 of 8 Type 2, and 30 ± 6 pA in 4 of 15 Type 3 non-serotonergic neurons), yet evoked currents of 77.9 ± 9.6 pA in all four LC neurons tested (Amy Jongeling and Donna Hammond, unpublished observations). These data indicate that the limited response of RVM neurons to DAMGO is a function of the tissue, and not the drug.

To further determine whether the limited effect of DAMGO in RVM neurons was due to suboptimal recording conditions (e.g. rundown), low levels of $G_{i/o}$ in RVM neurons, or an absence of G-protein regulated inwardly-rectifying K^+ channels (Kir3) channels, the effects of a GABA_B receptor agonist were examined. Like DAMGO, baclofen couples to $G_{i/o}$ and modulates conductance at Kir3 [2,31,35]. Unlike DAMGO, baclofen produced large, outward currents in RVM neurons (Fig. 4A,C). Importantly, of 11 non-serotonergic and eight serotonergic neurons that did not respond to a maximally effective concentration of DAMGO (1 or 3 μ M), nine (81.8%) and six (75%) responded to 3 or 10 μ M baclofen, respectively (e.g. Fig. 4C). Every neuron that responded to DAMGO, also responded to baclofen (e.g. Fig. 4A). Collectively, these data confirm that the paucity of DAMGO-responsive neurons in the RVM is not due to poor recording conditions, unhealthy neurons, low levels of $G_{i/o}$ or an absence of Kir3 channels in these neurons.

Persistent, but not Acute Inflammatory Nociception Increases the Proportion of Non-serotonergic Neurons that Respond to DAMGO

In control rats, DAMGO produced a concentration-dependent increase in the percentage of non-serotonergic neurons that responded with an EC_{50} (95% CL) of 0.3 (0.2 – 0.7) μ M and an E_{max} of $24.5 \pm 3.0\%$ (Fig. 5A). Four days after CFA, DAMGO was significantly more efficacious as evidenced by a shift in its concentration-response curve to the left and upward. The E_{max} of DAMGO increased to $52.5 \pm 3.8\%$, while its EC_{50} was unchanged [0.3 (0.2 – 0.4) μ M] (Fig. 5A). Of note, the amplitude of the current produced by DAMGO four days after CFA did not differ from that in control rats (Fig. 5C). A shorter period of inflammation, four hrs, did not increase the percentage of non-serotonergic neurons that responded to 1-3 μ M DAMGO (14 of 66 neurons) compared to control rats (15 of 62 neurons; $P > 0.8$; Fisher's Exact Test). It also did not increase the amplitude of the current evoked by DAMGO (22.4 ± 2.7 pA).

Persistent, but not Acute Inflammatory Nociception Also Increases the Proportion of Serotonergic Neurons that Respond to DAMGO

Bath application of DAMGO also produced a concentration-dependent increase in the percentage of non-serotonergic neurons that responded in control rats (Fig. 5B). The EC_{50} (95% CL) was 0.5 (0.4 – 0.6) μ M and its E_{max} was $27.6 \pm 1.2\%$. Again, the amplitude of the current was independent of concentration (Fig. 5D). Four days after CFA, the concentration-response curve for DAMGO was shifted to the left and upward, consistent with an increase in efficacy (Fig. 5B). E_{max} was increased to $51.0 \pm 1.5\%$, while the EC_{50} (95% CL) was unchanged from control [0.5 (0.4 – 0.6) μ M]. The amplitude of the current produced by DAMGO in serotonergic neurons in CFA-treated rats did not differ from that of controls (Fig. 5D). A shorter period of inflammation, four hr, did not increase the percentage of serotonergic neurons that responded to 1-3 μ M DAMGO (7 of 24 neurons in CFA-treated vs 12 of 45 neurons in control; $P > 0.9$; Fisher's Exact Text). The amplitude of the outward current produced by DAMGO was also unchanged four hrs after CFA (22.3 ± 5.3 pA).

The Potentiation of DAMGO's Effect is Neuron Specific

A secondary analysis was conducted to compare the responsiveness of the different types of neurons in control (saline and naïve), four-hr and four-day CFA-treated rats. Neurons were categorized by type, neurotransmitter content, and responsiveness to 1-3 μ M DAMGO. With respect to serotonergic neurons (Fig. 6A), the percentage of Type 1 neurons that responded to DAMGO was unchanged either four hrs or four days after CFA ($P > 0.8$, Fisher Freeman Halton Exact Test). The percentage of Type 2 neurons that responded to DAMGO was similarly unchanged four days after CFA ($P > 0.6$). No conclusions could be made about Type 2 neurons four hrs after CFA because only two such neurons (~10% of the total) were encountered. In contrast, the percentage of Type 3 serotonergic neurons that responded to DAMGO dramatically increased four days after CFA treatment ($P < 0.01$). The increase observed four hrs after CFA did not achieve statistical significance ($P = 0.069$).

With respect to non-serotonergic neurons (Fig. 6B), the percentage of DAMGO-responsive Type 1 neurons appeared to be diminished, but the decrease was not statistically significant ($P > 0.2$ for either 4 hr or 4 days). In contrast, the percentage of Type 2 non-serotonergic neurons that responded to DAMGO increased dramatically four days after CFA ($P < 0.01$). The percentage of Type 3 non-serotonergic neurons that responded to DAMGO was unchanged either four hrs or four days after CFA ($P > 0.2$ for each).

Persistent Inflammatory Nociception Does Not Enhance the Effects of another G Protein Coupled Receptor Agonist

Baclofen couples to Kir3 and Gi/o proteins as does DAMGO [2,31,35]. If persistent inflammatory nociception lead to an increase in the number of Kir3 or Gi/o proteins, then it could be expected that the effects of baclofen would also be enhanced four days after CFA. This was not the case. The potency and efficacy of baclofen to increase the percentage of responsive non-serotonergic neurons and current amplitude were similar in control and CFA-treated rats (Fig. 7A,C). The EC₅₀ (95% CL) of baclofen for number of responsive neurons was 1.7 (1.0 – 2.9) μ M in control rats compared to 2.2 (1.5 – 3.5) μ M in CFA-treated rats ($P > 0.4$). Similarly, the EC₅₀ (95% CL) of baclofen to produce 25 pA of outward current was 2.4 (0.7 – 4.2) μ M in control rats compared to 1.5 (1.2 – 2.9) μ M in CFA-treated rats ($P > 0.2$). The three different types of non-serotonergic neurons did not differ in their responsiveness to baclofen in either control or CFA-treated rats (data not shown).

Persistent inflammatory nociception also did not alter the concentration-response curves for baclofen in serotonergic RVM neurons in CFA-treated rats (Fig. 7B,D). The EC₅₀ (95% CL) of baclofen for number of responsive serotonergic neurons was 2.1 (0.4 – 12.3) μ M in control rats compared to 2.2 (0.51 – 6.82) μ M in CFA-treated rats. The EC₅₀ (95% CL) for baclofen to produce 25 pA of outward current was 1.6 (n.d. - 3.7) μ M in control rats compared to 1.8 (n.d. – 4.7) μ M in CFA-treated rats ($P > 0.2$). Lower CL could not be determined reliably because few data points fell below 25 pA at the lowest concentration tested.

DISCUSSION

This study is the first to document that persistent inflammatory nociception alters the passive membrane and action potential properties and the spontaneous discharge of specific populations of spinally projecting RVM neurons. Extracellular recording studies determined that NEUTRAL cells can transition into ON or OFF cells after injection of CFA [39] and that ON cell activity increases after mustard oil [27], but did not determine neurotransmitter content or axonal projections. The present findings indicate that, with the exception of Type 2 non-serotonergic and Type 3 serotonergic neurons, the intrinsic membrane properties of most spinally projecting RVM neurons are unaffected by persistent inflammatory nociception. Further study of the molecular basis and functional ramifications of the changes observed in these two populations may provide important insights into brainstem mechanisms that sustain persistent pain.

This study is also the first to examine the neuronal populations that could mediate the enhanced antinociceptive and anti-hyperalgesic effects of MOPr agonists in the RVM after CFA injection. *Mu* opioid receptors in the RVM are implicated in functions other than nociception [36,37]. Thus, opioid responsiveness is not definitive evidence for a role of a neuron in nociception, just as the absence of opioid responsiveness does not definitively exclude a role in nociception. Here, however, the emphasis was on identification of neurons in which the postsynaptic effects of DAMGO changed in a manner that was concordant with the time-dependent enhancement of its antinociceptive and anti-hyperalgesic effects in CFA-treated rats. Enhancement of MOPr-mediated antinociception and anti-hyperalgesia in the RVM is not evident until four days after CFA [23]. The finding that the postsynaptic effects of DAMGO were enhanced four days, but not four hours after CFA mirrors the behavioral results.

There was a striking concordance between the two populations of neurons whose electrophysiological properties were altered by persistent inflammatory nociception, and

those in which the effects of DAMGO were enhanced. Prolonged inflammation increased the percentage of Type 2 non-serotonergic neurons that were responsive to DAMGO. These same neurons also exhibited a more hyperpolarized resting membrane potential after CFA. In the absence of injury, Type 2 non-serotonergic neurons receive only weak excitatory glutamatergic input [48]. However, four days after CFA, excitatory glutamatergic input to these neurons is dramatically increased in a neurokinin-1 receptor dependent manner [48]. Since antagonism of neurokinin-1 receptors in the RVM reverses the thermal hyperalgesia induced by CFA [21], we have proposed that Type 2 spinally projecting non-serotonergic RVM neurons correspond to pain facilitatory neurons. An enhanced ability of DAMGO to suppress the excitability of or excitatory drive to these neurons would be consistent with its enhanced anti-hyperalgesic and antinociceptive effects four days after CFA.

The role of serotonergic RVM neurons in opioid-mediated analgesia remains controversial. Intrathecal administration of serotonin receptor antagonists attenuates the antinociceptive effects of MOPr agonists administered systemically or supraspinally [25,26,47] [but see 40]. Type 3 serotonergic neurons in this study may correspond to NEUTRAL cells, regularly discharging serotonergic neurons that are unresponsive to noxious stimulation and largely unaffected by MOPr agonists [4,13]. Type 3 serotonergic neurons may therefore not play an important role in the antinociceptive effects of MOPr agonists in acute models. However, after CFA, the spontaneous discharge of these neurons increases, as does the percentage that responds to DAMGO. These findings are consistent with the idea that serotonergic Type 3 neurons are pain facilitatory neurons whose activity increases after injury [44], and that their suppression contributes to the enhanced anti-hyperalgesic or antinociceptive effects of DAMGO.

The increase in DAMGO responsiveness is unlikely to be secondary to a “shift” in cell type in CFA-treated rats. All three neuron types were present in similar proportions in both treatment groups and, with few exceptions, no change in properties. What properties did change would not affect the categorization of the neurons. Had Type 3 (regularly firing) or Type 1 (sporadically firing) neurons become quiescent Type 2 neurons, the membrane resistance of Type 2 neurons in CFA-treated rats would have been larger than in control rats. At the same time, the capacitance of Type 2 neurons in CFA-treated rats would have decreased. Conversely, had Type 2 neurons become Type 1 or Type 3 neurons, a decrease in the membrane resistance and an increase in the capacitance of either Type 1 or Type 3 neurons would have occurred. Such changes were not observed making it unlikely that the increase in DAMGO responsiveness in Type 2 non-serotonergic neurons is due to a shift in neuron type. More difficult to detect or exclude is a possible switch between Type 1 and Type 3 neurons, whose passive membrane and action potential characteristics are similar. With respect to non-serotonergic neurons, the percentage of Type 1 DAMGO responsive neurons was marginally decreased while the percentage of Type 3 DAMGO responsive neurons was marginally increased. Neither change was significant. This situation could occur if a proportion of Type 3 neurons began to fire irregularly and assumed a Type 1 phenotype while a proportion of Type 1 neurons began to fire regularly and assumed a Type 3 phenotype. With respect to serotonergic neurons, only Type 3 neurons showed a significant increase in DAMGO-responsiveness. The DAMGO-responsiveness of Type 1 and Type 2 serotonergic neurons did not change. Had there been a shift of Type 1 or Type 2 serotonergic neurons into Type 3 neurons, capacitance should have either remained the same or increased in CFA-treated rats. Instead, it decreased in Type 3 serotonergic neurons.

The increase in the percentage of DAMGO responsive neurons in CFA-treated rats is also not secondary to an increase in current amplitude. Rapid desensitization can truncate the amplitude of DAMGO-induced currents and limit the ability to detect an increase in amplitude. However, we have seen no evidence for rapid desensitization in RVM neurons

(Fig. 7 of [50]; Fig. 4A, B). DAMGO and [Met⁵]enkephalin produced smaller currents in RVM neurons than in LC neurons of rats of the same age. It is well-established that efficacy is tissue-specific and that the efficiency of G-protein coupling to MOPr varies considerably among nuclei [32].

Type 3 serotonergic neurons exhibited an increased firing rate and a decrease in mean capacitance. An increase in neuronal activity is associated with an increase in the activity of TrpH, but levels of protein are not necessarily increased [11]. However, CFA treatment increases levels of brain derived neurotrophic factor in the RVM [19]. Brain derived neurotrophic factor can rapidly increase levels of TrpH mRNA and protein [42], and may play a critical role in determination of serotonergic phenotype [12]. At present, it cannot be determined whether the decrease in capacitance results from an increase in TrpH protein and an enhanced ability to immunohistochemically detect Type 3 serotonergic neurons, or whether it reflects a phenotypic change and the appearance of a novel population of serotonergic neurons that express the MOPr.

DAMGO and baclofen each couple to G_{i/o} and increase conductance at Kir channels [2,31,35]. In the LC, MOPr and GABA_B receptors exhibit heterologous desensitization suggesting they share a pool of G_{i/o} proteins or Kir channels [2]. Neither the percentage of baclofen-responsive neurons, nor the amplitude of the outward currents produced by baclofen increased in CFA-treated rats. The effects of baclofen were not enhanced even in neurons that co-expressed MOPr and GABA_B receptors and that could have shared access to G_{i/o} or Kir. Thus, the mechanism by which the postsynaptic effects of DAMGO are enhanced in RVM neurons probably does not involve an increase in available G_{i/o} proteins or Kir channels. Rather, the effect is likely to reflect mechanisms that are specific to the MOPr. Had inflammatory nociception selectively increased the coupling of MOPr to G_{i/o}, an increase in the amplitude of the currents induced by DAMGO would have been expected. This was not the case. Other possibilities that merit investigation include increased trafficking of MOPr to the plasma membrane due to a decrease in levels of β -arrestin 2 or dephosphorylation of Ser/Thr residues in the receptor.

These results provide important new evidence that persistent inflammatory nociception alters the electrophysiological properties and responsiveness of specific populations of spinally projecting RVM neurons. The concordance between the neurons in which changes occurred and those in which responsiveness to DAMGO was increased was striking, and lead us to propose that these may represent two different populations of bulbospinal pain facilitatory neurons. A recent report indicates that bulbospinal pain facilitatory pathways predominate in the RVM before age P25 [22]. Thus, this preparation could be biased towards the study of pain facilitatory neurons and not optimal for studies of pain inhibitory neurons. Regardless, these findings provide new evidence that persistent inflammatory nociception in the neonate has consequences for opioid action in the brainstem. Finally, these experiments addressed the postsynaptic actions of DAMGO on spinally projecting neurons in the RVM. They do not exclude an increase in the potency or efficacy of MOPr agonists to presynaptically suppress excitatory drive to pain facilitatory RVM neurons as an additional mechanism by which the antinociceptive and anti-hyperalgesic effects of MOPr agonists are enhanced under conditions of persistent inflammatory nociception.

Summary

Persistent inflammatory nociception enhances the postsynaptic effects of a mu opioid receptor agonist in specific populations of spinally projecting neurons in the rostral ventromedial medulla

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by R01 DA06736 to D.L.H. We thank Stephanie White, Adam Simonsen, and Johann Cutkomp for excellent technical assistance. The authors of this manuscript have no conflict of interest to report.

REFERENCES

- [1]. Beck SG, Pan YZ, Akanwa AC, Kirby LG. Median and dorsal raphe neurons are not electrophysiologically identical. *J Neurophysiol* 2004;91:994–1005. [PubMed: 14573555]
- [2]. Blanchet C, Luscher C. Desensitization of mu-opioid receptor-evoked potassium currents: initiation at the receptor, expression at the effector. *Proc Natl Acad Sci U S A* 2002;99:4674–4679. [PubMed: 11917119]
- [3]. Carlson JD, Maire JJ, Martenson ME, Heinricher MM. Sensitization of pain-modulating neurons in the rostral ventromedial medulla after peripheral nerve injury. *J Neurosci* 2007;27:13222–13231. [PubMed: 18045916]
- [4]. Chiang CY, Pan ZZ. Differential responses of serotonergic and non-serotonergic neurons in nucleus raphe magnus to systemic morphine in rats. *Brain Res* 1985;337:146–150. [PubMed: 4005602]
- [5]. Eckert WA 3rd, Willcockson HH, Light AR. Interference of biocytin with opioid-evoked hyperpolarization and membrane properties of rat spinal substantia gelatinosa neurons. *Neurosci Lett* 2001;297:117–120. [PubMed: 11121884]
- [6]. Fields HL, Vanegas H, Hentall ID, Zorman G. Evidence that disinhibition of brain stem neurones contributes to morphine analgesia. *Nature* 1983;306:684–686. [PubMed: 6656868]
- [7]. Finney, DJ. *Statistical Method in Biological Assay*. Hafner Publishing Co.; New York: 1964.
- [8]. Fitzgerald M. Developmental biology of inflammatory pain. *Br J Anaesth* 1995;75:177–185. [PubMed: 7577251]
- [9]. Fitzgerald M, Koltzenburg M. The functional development of descending inhibitory pathways in the dorsolateral funiculus of the newborn rat spinal cord. *Brain Res* 1986;389:261–270. [PubMed: 3948011]
- [10]. Freeman GH, Halton JH. Note on exact treatment of contingency, goodness of fit, and other problems of significance. *Biometrika* 1951;38:141–149. [PubMed: 14848119]
- [11]. Fuller RW, Steinberg M. Regulation of enzymes that synthesize neurotransmitter monoamines. *Adv Enzyme Regul* 1976;14:347–390. [PubMed: 9790]
- [12]. Galter D, Unsicker K. Brain-derived neurotrophic factor and trkB are essential for cAMP-mediated induction of the serotonergic neuronal phenotype. *J Neurosci Res* 2000;61:295–301. [PubMed: 10900076]
- [13]. Gao K, Chen DO, Genzen JR, Mason P. Activation of serotonergic neurons in the raphe magnus is not necessary for morphine analgesia. *J Neurosci* 1998;18:1860–1868. [PubMed: 9465010]
- [14]. Gilbert AK, Franklin KB. GABAergic modulation of descending inhibitory systems from the rostral ventromedial medulla (RVM). Dose-response analysis of nociception and neurological deficits. *Pain* 2001;90:25–36. [PubMed: 11166967]
- [15]. Gilbert M, Stelzner DJ. The development of descending and dorsal root connections in the lumbosacral spinal cord of the postnatal rat. *J Comp Neurol* 1979;184:821–838. [PubMed: 422765]
- [16]. Goncalves L, Almeida A, Pertovaara A. Pronociceptive changes in response properties of rostroventromedial medullary neurons in a rat model of peripheral neuropathy. *Eur J Neurosci* 2007;26:2188–2195. [PubMed: 17892482]
- [17]. Guan Y, Guo W, Zou S-P, Dubner R, Ren K. Inflammation-induced upregulation of AMPA receptor subunit expression in brain stem pain modulatory circuitry. *Pain* 2003;104:401–413. [PubMed: 12855351]

- [18]. Guan Y, Terayama R, Dubner R, Ren K. Plasticity in excitatory amino acid receptor-mediated descending pain modulation after inflammation. *J Pharmacol Exp Ther* 2002;300:513–520. [PubMed: 11805211]
- [19]. Guo W, Robbins MT, Wei F, Zou S, Dubner R, Ren K. Supraspinal brain-derived neurotrophic factor signaling: a novel mechanism for descending pain facilitation. *J Neurosci* 2006;26:126–137. [PubMed: 16399679]
- [20]. Hama AT, Fritschy J-M, Hammond DL. Differential distribution of GABA_A receptor subunits on bulbospinal serotonergic and nonserotonergic neurons of the ventromedial medulla of the rat. *J Comp Neurol* 1997;384:337–348. [PubMed: 9254031]
- [21]. Hamity MV, White SR, Hammond DL. Effects of neurokinin-1 receptor agonism and antagonism in the rostral ventromedial medulla of rats with acute or persistent inflammatory nociception. *Neuroscience*. 2009
- [22]. Hathway GJ, Koch S, Low L, Fitzgerald M. The changing balance of brainstem-spinal cord modulation of pain processing over the first weeks of rat postnatal life. *J Physiol* 2009;587:2927–2935. [PubMed: 19403624]
- [23]. Hurley RW, Hammond DL. The analgesic effects of supraspinal μ and δ opioid receptor agonists are potentiated during persistent inflammation. *J Neurosci* 2000;20:1249–1259. [PubMed: 10648729]
- [24]. Hurley RW, Hammond DL. Contribution of endogenous enkephalins to the enhanced analgesic effects of supraspinal μ opioid receptor agonists after inflammatory injury. *J Neurosci* 2001;21:2536–2545. [PubMed: 11264327]
- [25]. Jensen TS, Yaksh TL. Spinal monoamine and opiate systems partly mediate the antinociceptive effects produced by glutamate at brainstem sites. *Brain Res* 1984;321:287–289. [PubMed: 6149792]
- [26]. Jensen TS, Yaksh TL II. Examination of spinal monoamine receptors through which brainstem opiate-sensitive systems act in the rat. *Brain Res* 1986;363:114–127. [PubMed: 3004638]
- [27]. Kincaid W, Neubert MJ, Xu M, Kim CJ, Heinricher MM. Role for medullary pain facilitating neurons in secondary thermal hyperalgesia. *J Neurophysiol* 2006;95:33–41. [PubMed: 16192337]
- [28]. Kirby LG, Pernar L, Valentino RJ, Beck SG. Distinguishing characteristics of serotonin and non-serotonin-containing cells in the dorsal raphe nucleus: electrophysiological and immunohistochemical studies. *Neuroscience* 2003;116:669–683. [PubMed: 12573710]
- [29]. Leung CG, Mason P. Physiological survey of medullary raphe and magnocellular reticular neurons in the anesthetized rat. *J Neurophysiol* 1998;80:1630–1646. [PubMed: 9772227]
- [30]. Lin HH, Dun NJ. Post- and presynaptic GABA(B) receptor activation in neonatal rat rostral ventrolateral medulla neurons in vitro. *Neuroscience* 1998;86:211–220. [PubMed: 9692755]
- [31]. Luscher C, Jan LY, Stoffel M, Malenka RC, Nicoll RA. G protein-coupled inwardly rectifying K⁺ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. *Neuron* 1997;19:687–695. [PubMed: 9331358]
- [32]. Maher CE, Selley DE, Childers SR. Relationship of mu opioid receptor binding to activation of G-proteins in specific rat brain regions. *Biochem Pharmacol* 2000;59:1395–1401. [PubMed: 10751548]
- [33]. Marinelli S, Schnell SA, Hack SP, Christie MJ, Wessendorf MW, Vaughan CW. Serotonergic and nonserotonergic dorsal raphe neurons are pharmacologically and electrophysiologically heterogeneous. *J Neurophysiol* 2004;92:3532–3537. [PubMed: 15254076]
- [34]. Marinelli S, Vaughan CW, Schnell SA, Wessendorf MW, Christie MJ. Rostral ventromedial medulla neurons that project to the spinal cord express multiple opioid receptor phenotypes. *J Neurosci* 2002;22:10847–10855. [PubMed: 12486178]
- [35]. Marker CL, Lujan R, Colon J, Wickman K. Distinct populations of spinal cord lamina II interneurons expressing G-protein-gated potassium channels. *J Neurosci* 2006;26:12251–12259. [PubMed: 17122050]
- [36]. Mason P. Contributions of the medullary raphe and ventromedial reticular region to pain modulation and other homeostatic functions. *Annu Rev Neurosci* 2001;24:737–777. [PubMed: 11520917]

- [37]. Mason P. Ventromedial medulla: pain modulation and beyond. *J Comp Neurol* 2005;493:2–8. [PubMed: 16255004]
- [38]. Meek JL, Neff NH. Tryptophan 5-hydroxylase: approximation of half-life and rate of axonal transport. *J Neurochem* 1972;19:1519–1525. [PubMed: 4260826]
- [39]. Miki K, Zhou QQ, Guo W, Guan Y, Terayama R, Dubner R, Ren K. Changes in gene expression and neuronal phenotype in brain stem pain modulatory circuitry after inflammation. *J Neurophysiol* 2002;87:750–760. [PubMed: 11826044]
- [40]. Proudfit HK, Hammond DL. Alterations in nociceptive threshold and morphine-induced analgesia produced by intrathecally administered amine antagonists. *Brain Res* 1981;218:393–399. [PubMed: 6895046]
- [41]. Schepers RJ, Mahoney JL, Shippenberg TS. Inflammation-induced changes in rostral ventromedial medulla mu and kappa opioid receptor mediated antinociception. *Pain*. 2007
- [42]. Siuciak JA, Clark MS, Rind HB, Whittemore SR, Russo AF. BDNF induction of tryptophan hydroxylase mRNA levels in the rat brain. *J Neurosci Res* 1998;52:149–158. [PubMed: 9579405]
- [43]. Skagerberg G, Björklund A. Topographic principles in the spinal projections of serotonergic and non-serotonergic brainstem neurons in the rat. *Neuroscience* 1985;15:445–480. [PubMed: 4022334]
- [44]. Suzuki R, Rygh LJ, Dickenson AH. Bad news from the brain: descending 5-HT pathways that control spinal pain processing. *Trends Pharmacol Sci* 2004;25:613–617. [PubMed: 15530638]
- [45]. Terayama R, Dubner R, Ren K. The roles of NMDA receptor activation and nucleus reticularis gigantocellularis in the time-dependent changes in descending inhibition after inflammation. *Pain* 2002;97:171–181. [PubMed: 12031790]
- [46]. Weissmann D, Chamba G, Debure L, Rousset C, Richard F, Maitre M, Pujol JF. Variation of tryptophan-5-hydroxylase concentration in the rat raphe dorsalis nucleus after p-chlorophenylalanine administration. II. Anatomical distribution of the tryptophan-5-hydroxylase protein and regional variation of its turnover rate. *Brain Res* 1990;536:46–55. [PubMed: 2150774]
- [47]. Yaksh TL. Direct evidence that spinal serotonin and noradrenalin terminals mediate the spinal antinociceptive effects of morphine in the periaqueductal gray. *Brain Res* 1979;160:180–185. [PubMed: 581478]
- [48]. Zhang L, Hammond DL. Substance P enhances excitatory synaptic transmission on spinally projecting neurons in the rostral ventromedial medulla after inflammatory injury. *J Neurophysiol* 2009;102:1139–1151. [PubMed: 19494188]
- [49]. Zhang L, Jongeling AC, Hammond DL. Suitability of the retrograde tracer Dil for electrophysiological studies of brainstem neurons: adverse ramifications for G-protein coupled receptor agonists. *J Neurosci Methods* 2007;160:116–121. [PubMed: 17045656]
- [50]. Zhang L, Sykes KT, Buhler AV, Hammond DL. Electrophysiological heterogeneity of spinally projecting serotonergic and nonserotonergic neurons in the rostral ventromedial medulla. *J Neurophysiol* 2006;95:1853–1863. [PubMed: 16338998]

Figure 1. Distribution of DiI in the spinal cord of two representative rats. Panel A illustrates a section taken some distance from the location of the pledget. Panel B illustrates a section taken close to the location of the pledget.

Figure 2.

Low magnification images illustrating representative locations of RVM neurons from which recordings were made (green). Neurons immunoreactive for TrpH appear red. Panels A-C illustrate non-serotonergic neurons, while panel D illustrates a serotonergic neuron. Images are the projection of two adjacent 6- μ m confocal sections. In the course of recording, subsequent fixation and immunohistochemical processing the slices shrink by nearly 80%. Thus, the density of TrpH neurons in these images actually reflects the number present in 60 μ m of tissue.

Figure 3.

Characteristics of the spontaneous discharge of Type 1 and Type 3 non-serotonergic (A-C) and serotonergic (D-F) neurons in control and CFA-treated rats. ISI: interspike interval. The smaller the coefficient of variation of the ISI (CV_{ISI}), the more regular the discharge. Type 2 neurons are not spontaneously active and are therefore not illustrated here. * $P < 0.05$ ** $P < 0.01$, compared to corresponding control group by Student's t-test or Mann-Whitney as appropriate.

Figure 4.

(A) Representative recording from an RVM neuron that responded to both DAMGO and baclofen. The outward current produced by DAMGO was completely reversed by naloxone (NLX). This neuron was a Type 2 serotonergic neuron from a CFA-treated rat. (B) Representative recording from a Type 3 spinally projecting RVM neuron of a control rat. The outward current produced by [Met⁵]enkephalin was partially reversed by naloxone (NLX). (C) Representative recording from an RVM neuron illustrating the outward current induced by baclofen and its reversal by CGP35348. The antagonist concentration was lower than its estimated IC₅₀ of 50 μM [30], and thus the reversal was not complete. This recording was from a serotonergic Type 1 neuron in a saline-treated rat. Note that DAMGO was not effective in this neuron.

Figure 5.

Persistent inflammatory nociception enhances the postsynaptic effects of the *mu* opioid receptor agonist DAMGO in both serotonergic and non-serotonergic RVM neurons. Concentration response curves for DAMGO in (A) serotonergic and (B) non-serotonergic spinally projecting neurons in the RVM of control rats or rats four days after i.pl. injection of CFA. Ordinate for A-B: percentage of DiI-labeled RVM neurons in which DAMGO produced an outward current ≥ 10 pA. Ordinate for C-D: Outward current in pA. Abscissa: Concentration of DAMGO in μ M. Numbers above the symbols in panels A and B indicate the actual number of neurons that responded of the total tested at that concentration.

Symbols in panels C and D are the mean $\pm$ S.E.M. of determinations in the number of responsive rats indicated in panels A and B.

Figure 6.

The effect of DAMGO is enhanced in specific populations of spinally projecting RVM neurons in a neuron-specific manner. Percentage of spinally projecting serotonergic and non-serotonergic neurons responsive to either 1 or 3 μ M DAMGO as a function of cell type in control rats (open bars) or rats treated four hr (hatched bar) or four days (filled bar) with CFA. Numbers above the bars are the number of responsive neurons of the total tested with drug. * $P < 0.05$, ** $P < 0.01$ compared to control (Fisher's Freeman Halton Exact Test)

Figure 7.

Persistent inflammatory nociception does not enhance the postsynaptic effects of the GABA_B receptor agonist baclofen in either serotonergic or non-serotonergic RVM neurons. Concentration response curves for baclofen in (A, C) serotonergic and (B, D) non-serotonergic neurons in the RVM of control rats or rats four days after i.pl. injection of CFA. Ordinate for A-B: percentage of DiI-labeled RVM neurons in which baclofen produced an outward current ≥ 10 pA. Ordinate for C-D: Outward current in pA. Abscissa: Concentration of baclofen in μ M. Numbers above the symbols in panels A and B indicate the actual number of neurons that responded of the total tested at that concentration. Symbols in panels C and D are the mean $\pm$ S.E.M. of determinations in the number of responsive rats indicated in panels A and B.

Passive Membrane and Action Potential Properties in Spinally-Projecting Non-Serotonergic RVM Neurons in Control Rats and Four Days After CFA

Table 1

Cell Type	Treatment (N)	Memb. Capacit. (pF)	Memb. Resist. (MΩ)	RMP (mV)	APT (mV)	APA (mV)	APHW (ms)	Slow AHPA (mV)	APT to max AHPA (mV)	Fast AHP (mV)
Type 1	Control (28)	77.0 ^a (6.6)	584.8 ^a (79.8)	-48.1 ^a (0.6)	-37.1 ^{a,b} (0.5)	90.1 ^a (1.8)	0.74 ^a (0.03)	22.5 (0.8)	NA	18/28
	CFA (37)	84.1 ^d (7.4)	601.8 ^d (86.4)	-49.0 ^d (0.9)	-36.9 ^d (0.8)	83.4 ^d (2.1)	0.72 ^d (0.03)	22.5 (0.9)	NA	20/37
Type 2	Control (54)	102.2 ^b (4.4)	273.8 ^b (25.7)	-56.2 ^b (0.6)	-36.3 ^a (0.5)	83.1 ^b (1.4)	0.66 ^a (0.02)	NA	29.5 ^a (0.6)	39/54
	CFA (61)	97.5 ^d (4.6)	327.7 ^e (31.6)	-59.7 ^{e**} (1.2)	-35.0 ^e (0.5)	82.2 ^d (1.7)	0.67 ^d (0.02)	NA	29.8 ^d (1.3)	47/61
Type 3	Control (47)	88.4 ^{a,b} (6.6)	501.4 ^a (67.6)	NA	-38.8 ^b (0.6)	87.5 ^a (1.3)	0.73 ^a (0.03)	NA	27.5 ^a (0.6)	27/47
	CFA (47)	87.9 ^d (6.9)	521.7 ^d (79.6)	NA	-39.0 ^f (0.6)	87.0 ^d (1.3)	0.66 ^d (0.02)	NA	28.7 ^d (0.8)	33/47

Values are mean ± SEM. NA: Not applicable

* $P < 0.05$

** $P < 0.01$ compared to corresponding class of neuron in control rats.

^a comparisons among Type 1, 2 and 3 neurons in control rats

^b comparisons among Type 1, 2 and 3 neurons in control rats

^c comparisons among Type 1, 2 and 3 neurons in control rats

^d comparisons among Type 1, 2 and 3 neurons in CFA-treated rats.

^e comparisons among Type 1, 2 and 3 neurons in CFA-treated rats.

^f comparisons among Type 1, 2 and 3 neurons in CFA-treated rats.

Common superscripts indicate that the values for that measure do not differ among the different cell types as determined by one-way ANOVA or Kruskal-Wallis test. Abbreviations: RMP: resting membrane potential. APA: action potential amplitude. APT: action potential threshold. AHPW: action potential half width. AHP: afterhyperpolarization. Memb. Resist.: membrane resistance. Memb. Capacit.: membrane capacitance. In addition to saline-treated rats, control rats include naïve rats whose properties were previously reported [50].

Passive Membrane and Action Potential Properties in Spinally-Projecting Serotonergic RVM Neurons in Control Rats and Four Days After CFA

Table 2

Cell Type	Condition (N)	Memb. Capacit. (pF)	Memb. Resist. (MΩ)	RMP (mV)	APT (mV)	APA (mV)	APHW (ms)	Slow AHPA (mV)	APT to max AHP (mV)	Fast AHP
Type 1	Control (21)	64.9 ^a (4.3)	1007.6 ^a (91.3)	-50.6 ^a (0.7)	-35.9 ^a (0.6)	97.9 ^{a,b} (1.7)	1.13 ^a (0.04)	20.3 (0.8)	NA	2/21
	CFA (26)	61.0 ^d (4.7)	1134.4 ^d (103.7)	-51.8 ^c (0.8)	-36.7 ^d (0.6)	99.8 ^d (1.6)	1.07 ^c (0.03)	23.1 (1.3)	NA	1/26
Type 2	Control (19)	80.0 ^b (3.8)	519.4 ^b (48.0)	-65.1 ^b (2.0)	-37.4 ^a (0.7)	101.7 ^b (1.1)	1.04 ^a (0.03)	NA	29.8 ^a (1.1)	1/19
	CFA (23)	78.7 ^e (4.6)	649.7 ^e (86.8)	-64.2 ^d (2.1)	-37.7 ^d (0.5)	98.7 ^d (1.7)	0.99 ^c (0.04)	NA	29.7 ^c (1.2)	3/23
Type 3	Control (32)	57.2 ^a (4.3)	1120.3 ^a (115.7)	NA	-36.2 ^a (0.6)	94.4 ^a (1.6)	1.15 ^a (0.04)	NA	33.1 ^b (1.1)	3/32
	CFA (16)	39.4 ^{f**} (3.4)	942.2 ^{d,e} (138.7)	NA	-34.6 ^e (1.1)	88.4 ^e (2.5)	1.06 ^c (0.05)	NA	35.1 ^d (1.1)	5/16

Values are mean ± SEM. NA: Not applicable

* $P < 0.05$

** $P < 0.01$ compared to corresponding class of neuron in control rats.

^a comparisons among Type 1, 2 and 3 neurons in control rats

^b comparisons among Type 1, 2 and 3 neurons in control rats

^c comparisons among Type 1, 2 and 3 neurons in control rats

^d comparisons among Type 1, 2 and 3 neurons in CFA-treated rats.

^e comparisons among Type 1, 2 and 3 neurons in CFA-treated rats.

^f comparisons among Type 1, 2 and 3 neurons in CFA-treated rats.

Common superscripts indicate that the values for that measure do not differ among the different cell types as determined by one-way ANOVA or Kruskal-Wallis test. Abbreviations: RMP: resting membrane potential. APA: action potential amplitude. APT: action potential threshold. AHPW: action potential half width. AHP: afterhyperpolarization. Memb. Resist.: membrane resistance. Memb. Capacit.: membrane capacitance.