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Effects of local anesthetics on opioid inhibition of calcium current in rat dorsal root ganglion neurons

Hirochika Komai and Thomas S. McDowell*

Department of Anesthesiology, School of Medicine and Public Health, University of Wisconsin-Madison, United States

Abstract

Neuraxial analgesia is often provided using a mixture of local anesthetics and opioids. This combination of agents provides better pain relief and is generally associated with fewer side effects than when either drug is given alone. Local anesthetics have been shown to alter signaling of other G protein-coupled receptors, but little is known about their effect on opioid receptor signaling. Because opioids produce analgesia at least in part by inhibiting presynaptic Ca channels, we have evaluated the effects of tetracaine and bupivacaine on opioid-mediated inhibition of Ca channels in dorsal root ganglion neurons. The μ -opioid specific agonist DAMGO (1 μ M) inhibited Ca channels in both the absence and presence of tetracaine (50 or 100 μ M). However, the extent of DAMGO inhibition in the presence of both concentrations of tetracaine was less than that observed in the absence of tetracaine. DAMGO inhibition decreased from $39.2 \pm 24.4\%$ in control to $34.2 \pm 24.4\%$ with 50 μ M tetracaine ($n = 16$; $p < 0.05$), and from $40.5 \pm 19.6\%$ in control to $34.6 \pm 20.5\%$ with 100 μ M tetracaine ($n = 10$; $p < 0.05$). Similar results were seen with bupivacaine. Tetracaine also decreased the voltage-dependent facilitation of Ca channel currents when G proteins were activated by either DAMGO or the non-hydrolyzable GTP analogue (GTP γ S), suggesting that tetracaine weakens the interaction between G protein $\beta\gamma$ subunits and the Ca channel. Overall, these results suggest that local anesthetics decrease opioid inhibition of Ca channel activity by interfering with the GTP-mediated signal transduction between opioid receptors and Ca channels.

Keywords

Local anesthetics; Opioids; G proteins; DRG neurons; Sensory neurons; Calcium channels

Local anesthetics are often used in combination with opioids to provide postoperative neuraxial analgesia. While blockade of sodium channels in nociceptive neurons is the primary mechanism by which local anesthetics produce analgesia, the effects of local anesthetics are by no means limited to this. Local anesthetics also block both calcium and potassium channels [9,11,12], and have been shown to attenuate G protein-dependent inhibition of calcium current by somatostatin [13]. Tetracaine in particular has been shown to have more potent effects on G protein activity in vitro than lidocaine or bupivacaine [4]. Because opioids inhibit calcium current by a mechanism involving pertussis toxin-sensitive G_o proteins [10], it is possible that local anesthetics, through their effects on G proteins, may also interfere with opioid receptor signaling. We have examined this possibility by measuring the effects of the specific μ -opioid receptor agonist DAMGO and local anesthetics, singly and in combination, on calcium channel currents recorded from acutely isolated rat sensory neurons.

* Corresponding author at: Department of Anesthesiology, University of Wisconsin-Madison, B6/319 Clinical Sciences Center, 600 Highland Avenue, Madison, WI 53792-3272, United States. Tel.: +1 608 263 8698; fax: +1 608 263 0575. E-mail address: tsmcdowe@wisc.edu (T.S. McDowell).

Young Sprague–Dawley rats of either sex were deeply anesthetized with pentobarbital (200 mg/kg ip), and the spinal column was removed. This procedure caused minimal pain or discomfort to the animals, was in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and was approved by the Animal Care and Use Committee of the University of Wisconsin. Dorsal root ganglia were treated with collagenase (2 mg/ml) and trypsin (2.5 mg/ml) for 45 min at 35–37 °C and mechanically disrupted by trituration. DRG neurons were plated on polylysine-coated cover glass in DMEM:F12 medium containing calf serum (10%), penicillin (50 U/ml) and streptomycin (50 µg/ml) and maintained at 35 °C in an atmosphere containing 5% CO₂. Small neurons (diameter < 30 µm) were studied within 2 days after isolation.

Patch pipettes were pulled from borosilicate glass tubing. Whole cell currents were measured from DRG neurons at room temperature (21–25 °C) using an Axopatch 200B patch clamp amplifier and acquired using pCLAMP 9 software. Currents were filtered at 2 kHz and acquired at 10 kHz. For all cells ($n = 51$), average cell capacitance was 22 ± 6 pF and series resistance was 3 ± 1 MΩ. Seventy-five percent compensation of series resistance was applied. Whole cell configuration was achieved in a medium containing (in mM): NaCl, 130; KCl, 5; MgCl₂, 1; CaCl₂, 2; HEPES, 10; and glucose, 10 (pH 7.4 with NaOH). For most experiments, the external solution for the measurement of Ca channel activity contained (in mM): Tetraethylammonium chloride, 135; BaCl₂, 1; MgCl₂, 1; HEPES, 10; and glucose, 10 (pH 7.4 with CsOH). The pipette solution contained (in mM): CsCl, 80; CsOH, 40; EGTA, 10; HEPES, 10; MgATP, 2; LiGTP, 0.3; di-Tris phosphocreatine, 11 (pH 7.2 with CsOH). In some experiments, GTP was replaced by the same concentration of GTPγS. DAMGO [(D-Ala², NMePhe⁴, Gly-ol⁵) enkephalin] stock solution (1 mM) was prepared in distilled water, and frozen as 10 µl aliquots until use. DAMGO concentration was 1 µM in all experiments. Tetracaine and bupivacaine stock solutions (10 mM) were prepared in the external solution used for the measurement of Ca channel activity and kept frozen until use.

Ba currents were measured during 20 ms step depolarizations from a holding potential of –80 to –10 mV before and after a 40 ms depolarization (prepulse) to +70 mV. The currents measured before and after prepulse are designated as pre and post current, respectively. The area above the Ba current trace (total charge movement, pA × ms) was measured and the average value obtained during a given condition was used for further analysis. The area above the Ba current trace before the pre-pulse (pre current magnitude) and the ratio of the magnitudes of the post current and the pre currents (PPratio) are reported. Data were excluded if the magnitude of DAMGO inhibition in the absence of tetracaine was less than 10%, or if the magnitude of either the recovery pre current or the magnitude of the DAMGO inhibition was less than 80% or larger than 120% of the corresponding initial value.

Mean ± S.D. are shown. The differences between various treatments were analyzed by repeated measures analysis of variance followed by Tukey's or Dunnett's tests. When recovery values were significantly different from control, the effect of a treatment was compared to the average of initial control and washout value by paired *t*-test, or by ANOVA and Dunnett's test. Differences were considered significant when $p < 0.05$.

As reported by Sugiyama and Muteki [12], tetracaine decreased the Ba current magnitude in a concentration-dependent and reversible manner. In the presence of 50 µM tetracaine, the Ba current decreased to $76 \pm 7\%$ of the control value ($n = 16$; $p < 0.05$; Fig. 1), while in the presence of 100 µM tetracaine, the Ba current decreased to $53 \pm 6\%$ of control ($n = 10$, $p < 0.05$; Fig. 1).

DAMGO decreased the Ba current magnitude in both the absence and presence of tetracaine (Fig. 1). The combination of DAMGO and tetracaine (100 µM) decreased Ba current magnitude

to a greater extent than either DAMGO or tetracaine alone ($p < 0.05$). Tetracaine, however, significantly decreased the extent of DAMGO inhibition. In the presence of 50 μM tetracaine, DAMGO inhibition ($34.2 \pm 24.4\%$) was significantly lower than the DAMGO inhibition ($39.2 \pm 24.4\%$; $p < 0.05$) measured in the absence of the local anesthetic ($n = 16$). Washout of the local anesthetic resulted in partial restoration of DAMGO inhibition ($37.1 \pm 23.3\%$). The value in the presence of tetracaine was significantly lower than the average of control and washout (38.1 ± 23.8). Similarly, in separate experiments, DAMGO inhibition in the presence of 100 μM tetracaine ($34.6 \pm 20.5\%$) was significantly lower than the value ($40.5 \pm 19.6\%$; $p < 0.05$) obtained in the absence of the local anesthetic ($n = 10$). Washout of tetracaine restored the DAMGO inhibition to $39.0 \pm 18.6\%$, a value not significantly different from the control value. Ba current magnitude measured after washout of tetracaine (50 or 100 μM) was not significantly different from the corresponding initial control value.

Facilitation, a voltage-dependent reversal of G protein-mediated inhibition of Ca channels, is due to a decrease in the affinity of the $\beta\gamma$ subunits of the G protein for the Ca channel during the depolarization [3,8]. Facilitation is illustrated in Fig. 2, where Ba current traces recorded from one neuron before (pre) and after (post) a depolarizing voltage step are scaled and superimposed to illustrate differences in facilitation before, during, and after exposure to tetracaine (100 μM). Facilitation observed before DAMGO exposure (in the top row of the figure) reflects a low level of baseline G protein-mediated modulation of Ca channels. Tetracaine prevented the baseline facilitation and reduced facilitation in the presence of DAMGO.

The magnitude of facilitation was quantified by the post/pre (PP) ratio. We found that during DAMGO exposure, the magnitude of Ba current inhibition was closely correlated to the PP ratio (Fig. 3). This was true both in the control situation, and in the presence of tetracaine. Cumulative results for the effects of tetracaine on both DAMGO inhibition and the PP ratio are shown in Fig. 4.

Similar results were found using another local anesthetic, bupivacaine. At a concentration of 100 μM , bupivacaine reduced Ba current magnitude to a similar degree ($51 \pm 5\%$, $n = 6$) as 100 μM tetracaine (see Fig. 1C). The extent of DAMGO inhibition was reduced from $32.6 \pm 16.4\%$ in the absence, to $24.4 \pm 12.9\%$ in the presence of bupivacaine ($p < 0.05$). The baseline PP ratio was also decreased by bupivacaine, from 1.04 ± 0.02 to 0.99 ± 0.03 ($p < 0.05$). Recovery values for both the extent of DAMGO inhibition and the PP ratio were not different from control.

To confirm that changes in DAMGO-mediated inhibition and the PP ratio were not simply due to a decrease in Ba current magnitude, we repeated our experiments using a decreased extracellular concentration of Ba. When Ba concentration was decreased from 1 to 0.75 mM, Ba current magnitude was decreased to $76 \pm 6\%$ ($n = 9$) of control (Fig. 4), not significantly different (unpaired t -test) from that seen with tetracaine 50 μM . There were no changes in the magnitude of DAMGO inhibition, or in the PP ratios either before or during DAMGO exposure, in 0.75 mM Ba (Fig. 4).

The finding that tetracaine decreases baseline PP ratio in the absence of DAMGO stimulation, suggests that it acts at a site distal to opioid receptor activation. We confirmed this using the receptor-independent G protein activator GTP γ S. Inclusion of GTP γ S in the pipette produced rapid and sustained decreases in Ba current after patch rupture. As shown in Fig. 5, tetracaine produced concentration-dependent and reversible decreases in Ba current in the presence of GTP γ S similar in magnitude to those seen in control conditions (see Fig. 1). Due to the irreversible nature of the GTP γ S effect, we were unable to directly determine whether tetracaine could modulate the GTP γ S-mediated decrease in Ba current. However, tetracaine significantly

decreased the PP ratio during GTP γ S exposure, consistent with an impairment of G protein-mediated modulation of Ca channels by tetracaine.

The results of the present study indicate that local anesthetics decrease the extent of opioid inhibition of Ca channels in sensory neurons. The overall magnitude of the inhibition and the voltage-dependent facilitation, as measured by the PP ratio during DAMGO application, were both decreased. Local anesthetics also decreased the PP ratio measured in the absence of opioid agonist, as well as during receptor-independent G protein stimulation with GTP γ S. These findings suggest that local anesthetics act downstream of both opioid receptor and G protein activation, possibly by interfering with coupling between inhibitory $\beta\gamma$ subunits of the G-protein and Ca channels. This may be due to an interaction between the local anesthetics and the $\beta\gamma$ subunits, or to conformational changes in the Ca channel due to local anesthetic binding.

In sensory neurons, opioid receptors modulate voltage-gated Ca channels via G $_o$ -type G proteins [10]. On opioid agonist binding, the G protein $\beta\gamma$ subunits dissociate from the G α_o subunit, bind to Ca channels and rapidly modulate their function [5,7]. Strong depolarizations cause the $\beta\gamma$ subunits to temporarily dissociate from the Ca channel, a phenomenon known as voltage-dependent facilitation [14]. The PP ratio is a common measure of facilitation and a way to quantitate G protein-dependent inhibition of Ca currents. Indeed, we found that the PP ratio correlated closely with the magnitude of opioid inhibition. Because tetracaine decreased the PP ratio both in the absence of opioid agonist, and during receptor-independent G protein stimulation with GTP γ S, we suggest an action of tetracaine at the level of the $\beta\gamma$ subunits.

Local anesthetics have been shown to modulate the function of a variety of G proteins. However, most of these studies have focused on downstream effects of the α subunits of G proteins. For example, local anesthetics have been shown to inhibit G protein-mediated release of IP3-sensitive Ca stores in *Xenopus* oocytes, an effect mediated by the G α_q subunit [6]. In another study, lidocaine potentiated G α_i -mediated inhibition of cAMP induced by activation of adenosine A1 receptors expressed in CHO cells [1]. Local anesthetics may also have effects on opioid receptors and G α_o subunits in sensory neurons that we did not examine. Hagelüken et al. [4] reported that local anesthetics, notably tetracaine, increased both GTP γ S binding and GTP hydrolysis by G $_i$ and G $_o$ proteins. While increased GTP binding and increased GTP hydrolysis may both indicate G protein activation by tetracaine, excessive GTP hydrolysis could also lead to the rapid termination and thus inhibition of G protein-dependent responses.

Xiong et al. [13] reported that lidocaine at a concentration that had minimal effects on Ba current abolished the effect of somatostatin in anterior pituitary cells. Because somatostatin acts via G $_o$ in these cells, these results are consistent with our observations. However, we found relatively small effects even at high concentrations, compared to the almost complete loss of G protein-dependent inhibition of Ca channels in the study by Xiong et al. This may be due to differences in local anesthetics studied. Alternatively, differences between the preparations or the methods may also be responsible.

In conclusion, we found that tetracaine and bupivacaine produced small though statistically significant decreases in opioid inhibition of Ca channels. Clinically, it is well known that neuraxial analgesia is enhanced by the addition of opioids to local anesthetic solutions [2]. This is consistent with our finding that even with the small decrease in opioid efficacy, the combined effects of a local anesthetic and an opioid agonist on Ca channels are still more than the effect of local anesthetic or opioid alone.

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Fig 1.

Tetracaine reversibly decreased both Ca channel current magnitude and the DAMGO-induced inhibition of Ca channel currents. (A) Original Ba current traces recorded in the presence and absence of DAMGO (1 μ M), before (control), during (Tet (100 μ M)), and after (recovery) application of tetracaine. Results from 16 cells exposed to 50 μ M tetracaine are shown in (B), and the results of 10 cells exposed to 100 μ M tetracaine are shown in (C). Ba current (IBa) is expressed as a percentage of the control current magnitude for each cell; (*) indicates significant ($p < 0.05$; ANOVA) differences compared to the overall control values (30.3 ± 13.3 nA ms for 50 μ M tetracaine; 45.0 ± 18.2 nA ms for 100 μ M tetracaine), (†) indicates a

significant ($P < 0.05$; ANOVA) effect of DAMGO compared to the preceding measurement obtained in the absence of DAMGO under each condition.

Fig 2.

Tetracaine decreased the PP ratio measured both at baseline (before DAMGO; top panels) and in the presence of DAMGO (bottom panels). All traces in this figure were recorded from the same cell, under the conditions indicated. Each panel shows the pre current trace, recorded during a 20 ms voltage step -10 mV, superimposed with the post current trace, also recorded during a 20 ms voltage step to -10 mV, but after a 40 ms depolarizing pulse to $+70$ mV (not shown). Post current traces are marked by the filled circle. To illustrate changes, pre- and post current traces in each panel are scaled such that the last 5 ms of each pre current trace equals a magnitude of 1. Facilitation (an increase in the post compared to the pre current magnitude) is seen before (control) and after (recovery) tetracaine, both before (top panels) and during DAMGO (bottom panels). In the presence of tetracaine, facilitation was abolished before DAMGO, and reduced during DAMGO exposure.

Fig 3.

The degree of facilitation (PP ratio) during DAMGO exposure was positively correlated with the magnitude of DAMGO-induced inhibition of Ba current. The graphs show the results of individual experiments, before (open circles) and during (filled circles) tetracaine at either 50 μM (A) or 100 μM (B) concentrations. Lines connect results obtained during one experiment. Combining results in both the absence and presence of tetracaine, there was a significant linear correlation between the magnitude of DAMGO inhibition and the PP ratio. For tetracaine 50 μM ($n = 32$), $PP\ ratio = 0.021 \times DAMGO\ inhibition\ (\%) + 0.782$ ($r^2 = 0.862$, $n = 32$, $t = 13.7$, $p < 0.05$). For tetracaine 100 μM ($n = 20$), $PP\ ratio = 0.015 \times DAMGO\ inhibition\ (\%) + 0.828$ ($r^2 = 0.856$, $n = 20$, $t = 10.3$, $p < 0.05$).

Fig 4.

The effects of tetracaine on DAMGO-mediated inhibition and PP ratio are not due to a decrease in Ba current magnitude. Summary graphs comparing the effects of 50 and 100 μ M tetracaine (closed circles and closed squares, respectively) to the effects of a low external Ba concentration (0.75 mM Ba; open triangles). The 0.75 mM Ba solution led to a reduction in Ba current magnitude (IBa) that was similar to that seen with 50 μ M tetracaine (A). Despite this, there was no change in the magnitude of DAMGO inhibition (B), or in the PP ratio either at baseline (C) or during DAMGO exposure (D); (*) indicates significant ($p < 0.05$) difference from control values by ANOVA. When recovery was incomplete (significantly less than control; both PP ratios after 50 μ M tetracaine), (+) indicates a significant ($P < 0.05$) difference between tetracaine and the average of control and recovery values by t -test. Control current magnitude before 0.75 mM Ba was 31.0 ± 15.3 nA ms.

Fig 5.

Tetracaine reduced the PP ratio during GTP γ S-mediated stimulation. Inclusion of GTP γ S in the pipette led to rapid and stable decreases in Ba current magnitude (IBa) and increases in PP ratio typical of G protein-mediated inhibition of Ca channel currents. Exposure to 50 and 100 μ M tetracaine significantly and reversibly decreased the remaining Ba current when expressed as a percentage of the current magnitude recorded with GTP γ S alone (A; current magnitude in the presence of GTP γ S alone was 11.3 ± 6.4 nA ms). Importantly, tetracaine also decreased the magnitude of the PP ratio seen with GTP γ S stimulation (B), suggesting direct impairment of G protein-mediated Ca channel inhibition; (*) indicates significant ($p < 0.05$) difference from control values by ANOVA and Dunnett's *t*-test. When recovery was incomplete (significantly less than control), (†) indicates a significant ($P < 0.05$) difference of tetracaine compared to an average of control and recovery values by ANOVA and Dunnett's *t*-test.