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Effect of adjuvants on the action of local anesthetics in isolated rat sciatic nerves

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Abstract

Background and Objectives—There is increasing clinical use of adjuvant drugs to prolong the duration of local anesthetic-induced block of peripheral nerves. However, the mechanistic understanding regarding drug interactions between these compounds in the periphery is quite limited. Accordingly, we undertook this study to determine whether selected adjuvants are efficacious in blocking action potential propagation in peripheral nerves at concentrations used clinically, and whether these drugs influence peripheral nerve block produced by local anesthetics.

Methods—Isolated rat sciatic nerves were used to assess (1) the efficacy of buprenorphine, clonidine, dexamethasone, or midazolam, alone and in combination, on action potential propagation; and (2) their influence on the blocking actions of local anesthetics ropivacaine and lidocaine. Compound action potentials (CAPs) from A- and C-fibers were studied before and after drug application.

Results—At estimated clinical concentrations, neither buprenorphine nor dexamethasone affected either A- or C-waves of the CAP. Clonidine produced a small, but significant attenuation of the C-wave amplitude. Midazolam attenuated both A- and C-wave amplitudes, but with greater potency on the C-wave. The combination of clonidine, buprenorphine, and dexamethasone had no influence on the potency or duration of local anesthetic- or midazolam-induced block of A- and C-waves of the CAP.

Conclusions—These results suggest that the reported clinical efficacy of clonidine, buprenorphine, and dexamethasone influence the actions of local anesthetics via indirect mechanisms. Further identification of these indirect mechanisms may enable the development of novel approaches to achieve longer duration, modality-specific peripheral nerve block.

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Introduction

While there are a wide range of procedures for which short acting local anesthesia is ideal, it is becoming increasingly clear that long duration blocks are useful, particularly in the perioperative setting.^{1,2} One of several strategies that have been employed to increase the duration of local anesthetic block of peripheral nerves is based on the use of adjuvants that, when used in combination with local anesthetics, can prolong the duration of action. Epinephrine has been used most extensively in this capacity.³ Given the relatively limited efficacy of epinephrine, anesthesiologists have turned to additional compounds, including clonidine, buprenorphine, dexamethasone, and midazolam as single adjuvants or in combinations^{1,4–6} despite the fact that the mechanism(s) underlying the local anesthetic-prolonging actions of these compounds remain to be determined. Furthermore, the efficacy of these adjuvants used in combination with local anesthetics has encouraged anesthesiologists to use them in the absence of local anesthetics,^{1,4} in the hope that an optimal combination of adjuvants might have modality selectivity (ie, sensory instead of motor fiber block).

The purpose of this study was two-fold. First, to determine whether adjuvants now commonly used with local anesthetics in peripheral nerve blocks act directly on the peripheral nerve to block propagation of action potentials; and second, to determine if there is a direct effect of these adjuvants on the actions of local anesthetics in the block of propagated action potential in the isolated nerve. We hypothesized that (i) adjuvant drugs manifest anesthetic effects on their own at clinical concentrations, and (ii) these anesthetic effects are additive when drugs are combined. To test these hypotheses, we recorded compound action potentials (CAPs) from A-fibers (A-CAPs) and C-fibers (C-CAPs) in isolated rat sciatic nerves before and after application of adjuvants alone or in combination with the local anesthetic ropivacaine or lidocaine.

Methods

Animals

Adult male Sprague Dawley rats (275–350 g; Harlan Sprague Dawley, Indianapolis, Indiana) were used for the experiments. Rats were housed in an Association for Assessment and Accreditation of Laboratory Animal Care International-accredited animal care facility with unrestricted food and water on a 12:12-hr light-dark cycle until use. All procedures involving animals were reviewed and approved by the University of Pittsburgh Institutional Animal Care and Use Committee (Pittsburgh, Pennsylvania). Animal care and handling were in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

Drugs

We purchased all except 1 drug in preservative-free injectable solutions in the following concentrations: Clonidine HCl 0.1 mg/mL (Duraclon, Xanodyne Pharmaceuticals, Inc., Newport, Kentucky), buprenorphine HCl 0.3 mg/mL (Ben Venue Laboratories, Bedford, Ohio), dexamethasone sodium phosphate 10 mg/mL (preservative-free, with no benzyl alcohol; APP Pharmaceuticals, Schaumburg, Illinois), midazolam HCl (Ben Venue Laboratories) and ropivacaine HCl (Naropin, APP Pharmaceuticals) 5 mg/mL. Epinephrine and lidocaine HCl was purchased in powder form (Sigma-Aldrich, St. Louis, Missouri). The concentrations of buprenorphine, dexamethasone, epinephrine, midazolam, and ropivacaine used in the present study were based on estimation of concentrations that would be used clinically:⁶ 1 µg/mL (3.76 µM) for clonidine, 3 µg/mL (5.95 µM) for buprenorphine, 66.7 µg/mL (130 µM) for dexamethasone, 16.7 µg/mL (51.3 µM) for midazolam, and 2.5 mg/

mL (9.13 mM) for ropivacaine. Of note, dexamethasone is often used in a preparation made with 1% benzyl alcohol, and it is possible that influence of this adjuvant on the actions of local anesthetics is due to the alcohol rather than dexamethasone, per se. However, the preservative free form used in the present study is also used clinically, and there is evidence that this preparation can prolong the actions of local anesthetics.^{5,7} We therefore focused on the potential actions of dexamethasone rather than its preservative. The concentration of clonidine used is based on assumptions about infusion rates when the drug was used in clinical studies in combination with local anesthetics,^{8,9} as well as clinical observations regarding the sedative effects of clonidine. We therefore attempted to bracket this range in the experiments performed. The concentrations of lidocaine were based on those necessary to block voltage-gated Na⁺ channels in isolated tissue preparations,¹⁰ which are concentrations far below those used clinically. Adjuvants were tested at concentrations starting 2 to 3 orders of magnitude below the clinical values to at least 1 order of magnitude above the clinically relevant concentration. In the case of ropivacaine, we did not need to exceed the clinical dose because we were able to reach a maximum level of CAP attenuation at lower concentrations.

Recording CAPs from isolated sciatic nerves

Rats were anesthetized with an i.p. injection of either pentobarbital (60 mg/kg) or 1 mL/kg of a mixture of ketamine (55 mg/mL)/xylazine (5.5 mg/mL)/acepromazine (1.1 mg/mL). Sciatic nerves (~30 mm) were quickly dissected and immediately transferred to a container containing ice-cold Locke solution of the following composition (in mM): 136 NaCl, 5.6 KCl, 14.3 NaHCO₃, 1.2 NaH₂PO₄, 2.2 CaCl₂, 1.2 MgCl₂, 11 dextrose, equilibrated continuously with 95% O₂, 5% CO₂, pH 7.2 to 7.4. Nerves were trimmed of excess connective tissue and kept in ice-cold oxygenated Locke solution for at least 1 hour before use. One end of each nerve was laid over 2 platinum stimulating electrodes in the recording chamber. The central portion of the nerve, separated from the stimulating electrode by a grease-gap, was superfused continuously (2–5 mL/min) with oxygenated Locke solution at room temperature with and without drugs delivered via a gravity driven perfusion system. CAPs were recorded from the other end of the nerve with a glass suction electrode connected to the input stage of a differential preamplifier (0.1–10 kHz; WPI model DAM-80, Sarasota, Florida). CAPs were evoked with supramaximal electrical pulses 0.2 to 0.5 ms in duration, 0.05 Hz, filtered at 2 kHz, and sampled at 20 kHz. Voltage data were digitized via a CED 1401 Micro A/D converter and analyzed using CED Spike 2 version 5 for MS Windows (CED, Cambridge, England). Waveform data were rectified, averaged for 6 consecutive CAPs, and integrated to quantify A- and C-fiber components as area under the curve (AUC). The A-fiber deflection of the CAP (A-wave) was easily distinguished from that associated with the C-fiber deflection (C-wave) because of the time delay between the arrival of the 2 waves at the recording electrode. Specifically, we analyzed CAPs of A-fibers conducting between 15 and 80 m/s, and of C-fibers conducting slower than 1.5 m/s. Another wave with a conduction velocity consistent with A-delta fibers was present in many of the nerves studied. However, this wave was not included in subsequent analyses, both because of the variability in its amplitude and because the conduction velocity slowing caused by local anesthetics pushed this wave into the more slowing conducting C-wave, precluding a clear resolution of the impact of adjuvants and local anesthetics. It should be noted that an A-delta wave slowed by LA to the point that it was indistinguishable from the C-wave may have influenced our estimates of the potency of LA-induced block of the C-wave.

Statistics

The primary endpoints of this study were threefold: To determine 1) the potency and efficacy of drug-induced suppression of the compound action potential in the rat sciatic nerve, 2) the time-course of recovery of drug-induced suppression of the compound action

potential in the rat sciatic nerve, and 3) the influence of adjuvants on the potency and time course of recovery of local anesthetic-induced suppression of the compound action potential. To minimize the number of animals used in this study, both sciatic nerves were harvested from each rat and randomly assigned to different treatment groups. Each individual nerve was used for a single experiment, unless otherwise stated. Because there appeared to be no consistent differences between nerves studied from each animal relative to the total population of nerves studied, each nerve was treated as an independent observation. Thus, “n” represents the number of nerves rather than the number of animals. Nevertheless, to minimize the potential impact of a within-animal effect, nerves from at least 4 rats were used in each experimental group. To determine sample sizes, power analyses were performed with a two-way repeated measures ANOVA and a Student's t-test to determine the number of nerves needed for detecting a significant suppression of the CAP relative to control nerve followed over time and the number of nerves needed to detect a significant influence of adjuvants on the potency and time course of recovery of LA-induced CAP block. For the single-adjuvant concentration-response studies, we determined that a sample size of 6 was sufficient to enable us to detect a 25% suppression of the CAP with a power of 0.8 and alpha at 0.05. Similarly, for the LA studies with and without adjuvants, a sample size of 4 was sufficient to enable us to detect a change in potency of recovery greater than 25% with the same values for power and alpha. In several cases, additional nerves were used to increase our confidence in the negative results we obtained. AUC data from individual nerves were averaged for each group. Potency and efficacy of drug treatments were determined from concentration-response data fitted with a modified Hill equation of the form: $(1 - \text{CAP}_{\text{drug}}/\text{CAP}_{\text{ctrl}}) = ([\text{drug}]^{nH} E_{\text{max}})/([\text{drug}]^{nH} + \text{EC}_{50}^{nH})$, where EC_{50} is the concentration of drug needed block 50% of the CAP (a measure of potency), E_{max} is maximal fractional block of the CAP (a measure of efficacy) and nH is the Hill coefficient. A mixed-design two-way analysis of variance (time $\times$ treatment) was used to assess the impact of increasing concentrations of drug relative to control (choline) treated nerves. One-way ANOVA was used to assess differences in potency and efficacy between drug treatments. A students t-test was used to compare the impact of adjuvant drug combinations with local anesthetic or the impact midazolam had on the fractional block and/or the kinetics of the block (onset or recovery) relative to local anesthetic or to midazolam alone. All pooled data are expressed as a Fraction of Inhibition, which was determined for each nerve by the following equation: Fractional Inhibition = $1 - (\text{AUC}_{\text{Drug}}/\text{AUC}_{\text{Baseline}})$ where AUC_{Drug} was the area under the curve of the rectified CAP in the presence of drug and $\text{AUC}_{\text{Baseline}}$ was the area under the curve of the rectified CAP prior to the application of test agents.

Results

Concentration-Response curves of individual drugs

A total of 106 nerves were used in our experiments. Consistent with the results of previous studies, we observed a concentration-dependent block of both A- and C-waves of the sciatic nerve CAP by ropivacaine and lidocaine (Fig. 1). EC_{50} values for ropivacaine were 0.28 ± 0.04 mM and 0.18 ± 0.09 mM, for the A- and C-waves, respectively. Values for lidocaine were 0.28 ± 0.05 mM and 0.31 ± 0.7 for the A- and C-waves, respectively. Both drugs were fully efficacious, completely blocking both A- and C-waves.

Of the adjuvants tested, midazolam had the greatest efficacy in attenuating A- and C-waves of the CAP (Fig. 1). The effect of midazolam was concentration-dependent over the concentration range tested. Neither the A-wave nor the C-wave was fully blocked at the highest concentration tested (ie, 10 mM). Interestingly, in contrast to the A-wave, midazolam block of the C-wave appeared to be biphasic, with a high affinity component apparently saturating at ~35% block of the C-wave, and a lower affinity component that

seemed likely to enable complete block of the C-wave at greater concentrations of midazolam. Both components were significantly ($p = 0.03$) greater than the reduction of the C-wave observed in control nerves.

Previous data support the suggestion that clonidine has local anesthetic properties at high concentrations with an EC_{50} of ~ 2 and 0.45 mM for block of A- and C-waves.^{11,12} However, the preparation of clonidine used clinically is 0.38 mM out of the bottle, with a final concentration that is considerably lower (ie, 3.8 μ M as estimated previously^{1,6}). Consistent with these previous results, concentrations around those used clinically 0.3 to 30 μ M produced no detectable inhibition of the A-wave ($EC_{50} = 1.3$ μ M for $I_{max} = 0.01$) and a small (less than 50% of maximal) but significant ($p = 0.045$) inhibition of the C-wave. The EC_{50} for this effect was 5.3 μ M (Fig. 3). At non-neurotoxic concentrations approximating those used clinically, neither dexamethasone nor buprenorphine affected either A- or C-waves of the CAP (Fig. 2). As an additional control for the negative results obtained with dexamethasone and buprenorphine, we then tested epinephrine, an adjuvant that has been used more widely in clinical settings but which is thought to prolong the actions of local anesthetics via an extrinsic mechanism (ie, vasoconstriction) and therefore should not have a direct effect on the isolated nerve. Consistent with our expectations, epinephrine had no detectable influence on A- and C-waves (Fig. 2). Taken together, our results suggest the mechanisms underlying the action of these adjuvants are likely to be extrinsic to the peripheral axons.

Effects of co-application of clonidine-buprenorphine-dexamethasone on local anesthetic- or midazolam- induced inhibition of A- and C-CAPs

With little evidence that clonidine, dexamethasone, or buprenorphine at clinically relevant concentrations were efficacious in blocking A or C components of the CAP, we next assessed the impact of the combination of clonidine + dexamethasone + buprenorphine on the actions of local anesthetics and of midazolam. We first examined the potency and efficacy of local anesthetic-induced block by comparing the effects of an EC_{50} concentration of local anesthetic alone or in combination with supraclinical concentrations of clonidine (30 μ M), dexamethasone (1.3 mM), and buprenorphine (60 μ M). The combination of these 3 adjuvants alone had no detectable influence on the magnitude of either the A- or C-wave of the CAP, nor did it significantly influence the block produced by EC_{50} concentrations of ropivacaine (0.2 mM) or lidocaine (0.3 mM) (Fig. 3). Given the significant and potentially selective effect of midazolam on the C-wave, we next assessed the effect of a combination of clonidine + dexamethasone + buprenorphine on the block produced by midazolam (0.1 mM). As with the local anesthetics, these adjuvants had no detectable influence on the block produced by midazolam (Fig. 3). Finally, as a control for the negative results obtained with clonidine + dexamethasone + buprenorphine, we assessed the impact of epinephrine on the potency of lidocaine-induced block of the CAP. Consistent with our expectations, epinephrine had no detectable influence on the potency of lidocaine (Fig. 3).

Effect of the co-application of clonidine-buprenorphine-dexamethasone (CBD) on the time course of recovery from local anesthetic-induced block of the CAP

Because perineural adjuvants are primarily used with local anesthetics as a means to prolong the duration of local anesthetic-induced nerve block, we assessed the effect of the adjuvants on the time course of recovery from local anesthetic block. Preliminary results with ropivacaine suggested that the time to 50% recovery at well over 12 hrs was too long to have confidence in the viability of the preparation; therefore, we used the shorter acting local anesthetic lidocaine for these experiments. An EC_{50} concentration of lidocaine was applied to the isolated nerve alone or in combination with the 3 adjuvants (CBD). A stable block was produced for 10 minutes, after which all drugs were washed and recovery of the CAP

was monitored. The CBD adjuvants had no detectable influence on the time courses of recovery of either A or C-waves of the CAP when applied with the EC₅₀ concentration of lidocaine (Fig. 4). The time constants for recovery of the A-wave (41.91 ± 9.39 min for recovery from lidocaine alone and 41.12 ± 9.84 min for lidocaine + adjuvants) and C-wave (32.85 ± 9.57 min for recovery from lidocaine alone and 39.84 ± 17.12 min for lidocaine + adjuvants) did not differ significantly ($p > 0.05$ in both cases). To address the possibility that the lack of effect of adjuvants on recovery from lidocaine is concentration-dependent we repeated the time course of recovery experiments with a concentration of 3 mM lidocaine that completely blocks both A- and C-waves. Consistent with the results obtained with an IC₅₀ concentration of lidocaine, the CBD adjuvant combination had no significant ($p > 0.05$) influence on the time course of recovery from complete block of the sciatic nerve: the time constants of recovery were 24.41 ± 2.83 min and 56.27 ± 21.08 min for the A-waves and 28.49 ± 3.61 min and 48.83 ± 15.67 min for the C-waves for 3 mM lidocaine in the presence and absence of adjuvants, respectively. While not directly relevant to our central hypothesis, the CBD combination of adjuvants had no detectable influence on the rate of onset of local anesthetic-induced block (not shown). Finally, as a control for the negative results obtained with CBD on the time course of recovery from lidocaine-induced block, we assessed the effect of epinephrine on the time course of recovery from lidocaine-induced block of the CAP. Consistent with our expectations, epinephrine had no detectable influence on the time course of recovery of lidocaine-induced block: the time constants of recovery were 31.99 ± 3.36 min and 38.64 ± 9.72 min for A-waves and 28.13 ± 15.35 min and 33.43 ± 8.21 min for C-waves for 0.3 mM lidocaine in the presence and absence of epinephrine, respectively.

Discussion

The purpose of this study was to determine 1) whether the commonly-used adjuvants clonidine, buprenorphine, dexamethasone, and midazolam have efficacy in the block of action potential propagation at clinically-relevant concentrations by themselves, and 2) whether these drugs directly influence local anesthetic-induced block of action potential propagation. The principal findings from this study are threefold. First, of the adjuvants tested, only midazolam (and to a lesser extent clonidine) was efficacious in attenuating CAP propagation in the isolated sciatic nerve at clinically-relevant concentrations. The potency and efficacy of the block of the C-wave for both midazolam and clonidine were significantly greater than those of the A-wave. Second, the combination of clonidine, dexamethasone, and buprenorphine, each at a concentration 10 times that used clinically, had no influence on the potency of local anesthetic-induced block of the CAP or the potency and efficacy of midazolam-induced block of the CAP. Third, and most relevant to the understanding of the clinical use of these adjuvants, the combination of adjuvants did not increase the time course of recovery from lidocaine-induced block of the CAP. This last observation raises the possibility that adjuvants act indirectly to prolong the actions of local anesthetic-induced nerve block *in vivo*.

There are at least 3 aspects of the experimental design that may have impacted the results obtained and the conclusions drawn from them. First, all electrophysiological experiments were conducted at room temperature. This was done because we sought to analyze the impact of anesthetics and adjuvants on A- and C-waves in relative isolation, which is difficult to achieve at elevated (eg, body) temperatures, especially with relatively short nerves (<30 mm). If adjuvants influence the actions of local anesthetics by affecting the interaction between anesthetics and voltage-gated Na⁺ channels, and if this interaction were temperature-dependent, it is possible that by recording at room temperature we failed to detect such an interaction. Second, these studies were conducted on sciatic nerves isolated from the rat, and data that support the use of adjuvants to prolong the actions of local

anesthesia had been collected in humans. Thus, species differences, in particular those specific to voltage-gated Na⁺ channels, the primary target of local anesthetics, may have contributed to our failure to detect an influence of adjuvants. Third, we were forced to perform our “recovery” experiments with lidocaine rather than ropivacaine because the time course for the recovery from ropivacaine alone (ie, >12 hrs) precluded the ability to accurately measure an influence of adjuvants. It is therefore possible that we may have missed an influence of adjuvants that are specific to the longer-acting ropivacaine.

Results of the present study suggest effects of midazolam are due to a direct action on the peripheral nerve. While the actions of midazolam are not restricted to the gamma subunit containing A-type GABA (GABA_A) receptor,^{13,14} we are not aware of evidence to suggest that this compound has any influence on voltage-gated Na⁺ channel activity. Our own preliminary data on Na⁺ currents in isolated sensory neurons (data not shown) suggest that at the concentrations used in the present study, the blocking effects are not due to an action on Na⁺ channels. Furthermore, while there is evidence that GABA_A receptors are present¹⁵ and functional^{15,16} in peripheral nerve, the inability of midazolam to directly activate GABA_A receptors argues against a GABA_A receptor-mediated increase in chloride conductance as a mechanism for midazolam-induced block of the CAP. Of the known targets for midazolam, this leaves the peripheral benzodiazepine receptor as a potential mechanism of action, although additional data would be needed to both confirm this possibility and identify the link between the peripheral benzodiazepine receptor and CAP block.

One of the more interesting observations in the present study was the differential influence of midazolam on the A- and C-waves of the CAP. A selective C-fiber block would be ideal in the clinical setting, given that the majority of nociceptive signals, particularly those associated with tissue injury, are carried by C-fibers.¹⁷ Thus, such a selective block should result in the suppression of pain while leaving motor and proprioceptive fibers intact, thereby mitigating 1 of the major deleterious consequences of complete nerve block. While these results are intriguing, our previous data¹¹ suggest that midazolam is toxic to primary afferents at concentrations used clinically.¹ Identifying the mechanism of midazolam-induced block, however, may enable identification of other compounds that confer a selective C-fiber block in the absence of toxicity.

An isolated peripheral nerve *in vitro* preparation was used in the present study because we sought to determine whether the interaction between local anesthetics and adjuvants involved mechanisms intrinsic to the peripheral nerve. The failure to detect an influence in isolated nerves suggests that the nature of the interaction involves mechanisms extrinsic to the peripheral nerve. For example, clonidine may be acting at alpha-2 adrenergic receptors on sympathetic post-ganglionic terminals, or local vasculature, or via an “off target” mechanism, such as the hyperpolarization-activated cation current (I_h)¹⁸ in a cell type that is subsequently able to influence local anesthetic actions on the primary afferent. Similarly, dexamethasone may reduce inflammation associated with anesthetic administration, thereby indirectly prolonging anesthetic effects. Given the presence of opioid receptors in both local¹⁹ and recruited immune²⁰ cells, buprenorphine could be acting indirectly via a comparable mechanism. Future *in vivo* studies will be needed to explore these possibilities.

In summary, these results indicate that adjuvants reported to prolong the actions of local anesthetic-induced block of peripheral nerve do so by indirect mechanisms. Identification of these mechanisms may enable the development of novel approaches to both prolong the actions of local anesthetics and increase the therapeutic window of these compounds.

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Figure 1.

Traces from a representative recording session illustrating changes in rectified **A)** A-CAPs and **B)** C-CAPs, associated with increased ropivacaine concentrations shown in **C** and **D**. Lighter shades indicate greater ropivacaine concentrations (0.01 – 3 mM). Insert in **B** shows C-waveforms before rectification. **C)** Concentration-response curves for changes in A-CAPs quantified as area under the curve (AUC) for a given trace as a function of ropivacaine (black circles) or lidocaine (gray circles) concentration. Data are presented as mean $\pm$ SEM for each concentration, whereas black and gray diamonds represent the concentrations that correspond to EC_{50} values for ropivacaine (n=7) and lidocaine (n=5), respectively. **D)** Concentration-response curves for changes in C-CAP AUCs from the same nerves as in **C**. **E** and **F)** Concentration-response curves for midazolam A- and C-CAPs (n = 6).

Figure 2.

Traces from a representative recording session, illustrating changes in rectified **A)** A-CAPs and **B)** C-CAPs, associated with increasing clonidine concentrations. Lighter shades indicate greater concentrations (0.3 – 30 μ M) of clonidine. Insert in **B** shows C-waveforms before rectification. **C)** Concentration-response data for changes in A-CAP AUCs for clonidine (white), dexamethasone (black), and buprenorphine (gray). Data are presented as mean $\pm$ SEM for each concentration (clonidine n=5, dexamethasone n=6, buprenorphine n=5). **D)** Concentration-response data for changes in C-CAP AUCs from the same nerves as in **C**. **E and F)** Baseline levels of A-AUC (**E**) and C-AUC (**F**) over 2 hrs. **G)** Comparison of A-AUC maximal inhibition obtained from the last data points in (**C**) for each adjuvant and for epinephrine (n=3) to the baseline data (n=7), recorded for the same duration. **H)** Comparison of C-AUCs to baseline for the same nerves in **G**.

Figure 3.

Addition of adjuvants does not enhance local anesthetic- or midazolam-induced decreases in CAPs. **A and B**) Rectified A-CAP and C-CAP (inserts) traces before (solid lines) and after (dotted lines) 0.2 mM ropivacaine application in the absence (**A**) and presence (**B**) of clonidine-buprenorphine-dexamethasone (CBD) mixture. **C and D**) Drug-associated percentage decreases in **C**) A-AUCs and **D**) C-AUCs. CBD, n=6; R, ropivacaine alone, n=9; RCBD, ropivacaine and CBD mixture, n=9; L, lidocaine, n=4; LCBD, Lidocaine and CBD mixture, n=6; LE, Lidocaine and Epinephrine, n=3; M, midazolam, n=5; MCB, midazolam and CBD mixture, n=5.

Figure 4.

Presence of clonidine-buprenorphine-dexamethasone (CBD) does not influence wash-out kinetics of lidocaine (L). **A)** Changes in A-AUCs and **B)** C-AUCs plotted for 60 min after the start of the wash following 0.3 mM lidocaine (gray) or 0.3 mM lidocaine + CBD mixture (black) treatments. **C)** Changes in A-AUCs and **D)** C-AUCs plotted for 80 min after the start of the wash following 3 mM lidocaine (gray) or 3 mM lidocaine + CBD mixture (black) treatments. $P=NS$ for all comparisons.