Published in final edited form as:
Neurosci Lett. 2005 May 20; 380(1-2): 21–25.

Differential effects of bupivacaine and tetracaine on capsaicin-induced currents in dorsal root ganglion neurons

Hirochika Komai and **Thomas S. McDowell***
Department of Anesthesiology, University of Wisconsin Medical School, B6/319 Clinical Science Center, 600 Highland Avenue, Madison, WI 53792-3272, USA

Abstract

Capsaicin opens the TRPV1 channel, a cation channel that depolarizes and activates nociceptive neurons. Following this initial activation, neurons become desensitized to subsequent applications of capsaicin as well as to other noxious stimuli, a phenomenon attributed primarily to the entry of Ca^{2+} ions through the open TRPV1 channel. This ability of capsaicin to desensitize nociceptors has led to its use as an analgesic in the treatment of a variety of chronic pain states. Because treatment with capsaicin is initially quite painful, local anesthetics are sometimes used to block axonal conduction in nociceptive neurons and thus minimize pain. However, local anesthetics might also block TRPV1 and prevent the Ca^{2+} entry required for capsaicin-induced desensitization. We have studied the direct effect of local anesthetics on currents induced by capsaicin (1 μM) in acutely isolated rat dorsal root ganglion neurons using the whole cell patch clamp technique. At the highest concentration tested (1 mM), bupivacaine only moderately inhibited the capsaicin-induced current to 55 ± 27% of control (mean ± S.D.; $n = 12$, $p < 0.01$). Tetracaine (1 mM), on the other hand, enhanced the capsaicin-induced current to 151 ± 34% of control (mean ± S.D.; $n = 7$, $p < 0.01$). These results show that local anesthetics can be used to prevent the initial pain induced by application of capsaicin without abolishing, and perhaps even enhancing, its desensitizing actions.

Keywords

Capsaicin; TRPV1; VR1; Ionic currents; Dorsal root ganglion neuron; Sensory neuron; Local anesthetics

Capsaicin, the main pungent ingredient in chili peppers, elicits a sensation of burning pain by binding to and activating the TRPV1 channel on the afferent endings of heat-sensitive primary nociceptive neurons. TRPV1 is a nonspecific cation channel that allows Na^+ and Ca^{2+} ions to flow into the cell when it is open, thus depolarizing the cell and initiating action potentials in nociceptor axons. Repeated or prolonged applications of capsaicin cause desensitization of TRPV1 responses in vitro and degeneration of afferent nerve endings in vivo, both likely due to Ca^{2+} entering the cell through the TRPV1 channel [9]. After capsaicin administration, not only are neuronal responses to subsequent capsaicin challenges reduced, but responses to other noxious stimuli are reduced as well, providing the rationale for using capsaicin and its analogues as analgesics. Because administration of capsaicin is quite painful initially, however, local anesthetics are sometimes used before or in combination with capsaicin to reduce the initial pain while preserving desensitization. Although local anesthetics likely prevent pain in this setting by blocking voltage-gated Na^+ channels and thus preventing action potential propagation in nociceptor axons, there is indirect evidence that local anesthetics may also block TRPV1 channels [8]. If so, administration of local anesthetics along with capsaicin may

* Corresponding author. Tel.: +1 608 265 3186 (Lab)/1 608 263 8698 (Hospital); fax: +1 608 263 0737 (Lab)/1 608 263 0575 (Hospital).
E-mail address: tsmcdowe@wisc.edu (T.S. McDowell)

undermine the goal of inducing desensitization and neuronal degeneration since TRPV1 channels would not be fully activated. In the present study, we have examined the direct effects of bupivacaine and tetracaine on capsaicin-induced currents in acutely isolated sensory neurons.

Young Sprague–Dawley rats of either sex were deeply anesthetized with an intraperitoneal injection of pentobarbital (200 mg/kg), after which the spinal column was removed. All procedures were 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) or dispase (2 mg/ml) for 45 min at 35–37 °C. Following enzyme treatment, the ganglia were dissociated by trituration with fire-polished Pasteur pipettes. Sensory neurons were collected by low speed centrifugation, resuspended in DMEM:F12 (1:1) containing calf serum (10%), penicillin (50 U/ml) and streptomycin (50 μg/ml), and allowed to settle on cover glass coated with polylysine. Cells were maintained at 35 °C in a humidified incubator equilibrated with a gas mixture containing 5% CO_2. The cells were used within 2 days after isolation. Only small cells (diameter < 30 μm) were used.

Patch pipettes were pulled from borosilicate glass tubing. Whole cell currents were measured from dorsal root ganglia neurons at room temperature (21–25 °C) using an Axopatch 200B (Axon Instruments, Foster City, CA) patch clamp amplifier and acquired using pClamp 6 software (Axon Instruments, Foster City, CA). Currents were filtered at 1 kHz and acquired at 4 kHz. Cell capacitance and series resistance were read from the dials of the patch clamp amplifier after cancellation of the capacitative current transient obtained during a small depolarizing test pulse. Seventy percent compensation of series resistance was applied. Gigaseal formation and the whole cell configuration were achieved in a medium containing (in mM): NaCl, 130; KCl, 5; $MgCl_2$, 1; $CaCl_2$, 2; HEPES, 10; and glucose, 10 (pH 7.4 with NaOH). Based on the study of Koplas et al. [9], we used a Ca^{2+}-free external solution and a pipette solution containing the fast Ca^{2+} chelator BAPTA to minimize desensitization and allow repeated measurements of capsaicin-induced current in the same cell. The Ca^{2+}-free external solution contained (in mM): NaCl, 130; KCl, 5; $MgCl_2$, 5; EGTA, 1; HEPES, 10; and glucose, 10 (pH 7.4 with NaOH). The pipette solution contained (in mM): CsCl, 80; CsOH, 40; BAPTA, 10; HEPES, 10; MgATP, 2; LiGTP, 0.3; di-Tris phosphocreatine, 11 (pH 7.2 with CsOH). The membrane potential was maintained at −80 mV. To elicit the capsaicin-induced current, the cell was exposed first to the Ca^{2+}-free external solution and then switched by means of perfusion fast-step (Warner Instrument Corporation) to the Ca^{2+}-free external solution containing capsaicin for 2.5 s. After 1 min perfusion with the Ca^{2+}-free external solution, the cell was exposed to a Ca^{2+}-free external solution containing capsaicin and a local anesthetic for 2.5 s. After 1 min perfusion with the Ca^{2+}-free external solution, the reversibility of the local anesthetic effect was tested by measuring the current elicited by capsaicin in the absence of local anesthetic for 2.5 s. Thus, throughout the experimental period, the cells were not exposed to external medium containing Ca^{2+}. One concentration of a local anesthetic was tested in each cell. We report the peak magnitude of capsaicin-induced currents after subtraction of the mean holding current at −80 mV recorded before the application of capsaicin containing solution. The amplitude of capsaicin-induced current under control conditions widely varied among the cells, ranging from smaller than 1 nA to larger than 10 nA. Because of this, the effects of local anesthetics were studied by comparing the current in the presence of a local anesthetic to its own control measured with the same cell. The data obtained after washout of the local anesthetic were also compared to the initial control. Data were excluded when there was a progressive decrease in the current amplitude or when the recovery after washout of a drug was less than 50% or above 150% of the control. The effects of local anesthetics on current amplitude were analyzed by repeated-measures analysis of variance followed by Dunnett's *t* test for comparison against the initial control after log transformation of the data. The effects of local anesthetics on time to peak current were analyzed similarly but without log

transformation. Data are expressed as means ± S.D. Differences were considered significant when $p < 0.05$.

Capsaicin (1 μM) produced an inward current in most cells tested. Capsaicin-induced currents activated rapidly after switching to perfusate containing capsaicin, and decayed slowly after switching back to the control solution (see Fig. 1, left panel). When cells were exposed to perfusate containing bupivacaine and capsaicin, the magnitude of the capsaicin-induced currents was reduced. The extent of inhibition by bupivacaine increased as the concentration increased from 100 to 500 μM but showed no further increase in the presence of 1 mM bupivacaine (Figs. 1 and 2). At a concentration of 1 mM, bupivacaine reduced the current amplitude to 55 ± 27% of the peak control current measured before the end of the capsaicin application ($p < 0.01$, $n = 12$). The effect was reversible, as the magnitude of a subsequent capsaicin application in the absence of bupivacaine (recovery) was 110 ± 26% of control (NS).

At concentrations of bupivacaine above 100 μM, we often observed a rapidly developing secondary increase in the capsaicin-induced current that occurred after switching back to control perfusate which contained neither capsaicin nor bupivacaine, as seen in the middle panel of Fig. 1. After application of capsaicin and 1 mM bupivacaine, the peak amplitude of this secondary increase occurred at 5.4 ± 1.2 s after the start of the recording, clearly after changing back to control solution and significantly later than the peak of the control capsaicin current (3.9 ± 0.7 s; $p < 0.01$, $n = 12$). The magnitude of the peak of the secondary increase was 92 ± 32% of the peak of the control capsaicin-induced current (NS). In contrast, the maximal current measured during capsaicin and bupivacaine application occurred at 3.4 ± 0.3 s, not significantly different from control. We reasoned that the secondary increase in the current might be due to a rapid washout of bupivacaine after switching back to the control solution, while the effect of capsaicin would be expected to persist (see Fig. 1, left panel). We therefore conducted additional experiments in which cells were exposed to perfusate containing 1 mM bupivacaine after being exposed to capsaicin and bupivacaine (1 mM). As demonstrated in Fig. 3, the current magnitude was decreased during application of capsaicin and bupivacaine, but there was no secondary increase in current magnitude after switching to a solution containing bupivacaine but no capsaicin. Similar results were seen in four other cells.

Unlike bupivacaine, tetracaine increased the magnitude of the capsaicin-induced current (Figs. 4 and 5). This only occurred at the highest concentrations of tetracaine tested. In the presence of 1 mM tetracaine, for example, the maximum capsaicin-induced current was 151 ± 34% of control ($n = 7$, $p < 0.01$), while the time to the maximum current in the presence of tetracaine (4.2 ± 0.3 s) was not significantly different from the control value (4.0 ± 0.3 s). The current amplitude of a subsequent application of capsaicin without tetracaine was 101 ± 28% of control (NS), indicating that the effect of tetracaine was reversible. Tetracaine (1 mM) alone (without capsaicin) elicited minimal currents in some cells. A small (about 10%) inhibitory effect of 100 μM tetracaine was observed with a few cells, but the effects were not statistically significant. Tetracaine did not have a distinct secondary component upon removal as observed with bupivacaine.

The moderate inhibitory effect of bupivacaine and stimulating effect of tetracaine are consistent with the results of in vivo studies which show that local anesthetics do not prevent capsaicin-induced desensitization. Avelino et al. [1] demonstrated that lidocaine prevents noxious excitation induced by capsaicin as measured by Fos immunoreactivity but does not prevent desensitization to repeated capsaicin applications. They suggested that capsaicin-induced depolarization and subsequent Na^+ influx through the voltage-gated Na^+ channel is more important for excitation whereas calcium influx is the important process in desensitization. Craft and Porreca [3] showed that tetracaine attenuated the increase in nociceptive response (abdominal licking) induced by resiniferatoxin, a potent analog of capsaicin, but did not alter

and in some cases even enhanced the development of desensitization. These results may be at least partially explained by our finding that tetracaine increased capsaicin-induced currents, which would lead to greater influx of Ca^{2+} and greater desensitization.

Application of either capsaicin or heat activates TRPV1, which depolarizes the cell by allowing influx of Na^+ and Ca^{2+} ions through the TRPV1 channel. The depolarization activates both voltage-dependent Na^+ and Ca^{2+} currents, the latter leading to further increases in intracellular Ca^{2+}. In addition, intracellular Ca^{2+} may be released in response to capsaicin [4,10]. In rat dorsal root ganglia, the increase in intracellular Ca^{2+} induced by heat is sensitive to Na^+ removal (75% inhibition) and addition of nifedipine (62% inhibition) [7]. This suggests that about 1/3 of total Ca^{2+} increase involves Ca^{2+} influx through TRPV1 channels and about 2/3 involves influx through voltage-gated channels. On the other hand, with the peripheral sensory nerve terminals of rat eyes, Gover et al. [6] showed that Ca^{2+} increases induced by capsaicin (1 μM) are not sensitive to STX or nifedipine. Németh et al. [11] found that the liberation of substance P, calcitonin gene-related peptide and somatostatin from isolated rat tracheae induced by capsaicin (10 nM) was not inhibited by lidocaine (25 mM), TTX (1 μM), or ω-conotoxin GVIA. However, ω-agatoxin TK significantly inhibited and Cd^{2+} (200 μM) prevented capsaicin-induced neuropeptide release. The authors suggested that contribution of Q-type but not Nor P-type Ca channel is possible.

The effects of local anesthetics on ionic currents and intracellular Ca^{2+} levels in sensory neurons are complex. First, at high concentrations, local anesthetics themselves induce inward currents and depolarize cells. Thus, Vlachová et al. [12] found that procaine and bupivacaine excite nociceptive neurons that are sensitive to heat and capsaicin by inducing an inward current. Gold et al. [5] showed that lidocaine at high concentrations (>1–10 mM) depolarized rat sensory neurons and increased intracellular Ca^{2+} levels, the latter due to both an influx of extracellular Ca^{2+} and a release of Ca^{2+} from internal stores. In HEK293 cells, both lidocaine and prilocaine were found to increase intracellular Ca^{2+} concentrations, an effect that was not dependent on activation of TRPV1 channels [8]. We did not observe a consistent effect of local anesthetics on membrane currents.

On the other hand, local anesthetics have generally been found to have an inhibitory effect on capsaicin-induced currents. At concentrations of 10 mM, both lidocaine and prilocaine almost completely inhibited the increase in intracellular Ca^{2+} induced by capsaicin-induced activation of recombinant TRPV1 expressed in human embryonic kidney cells [8]. Procaine, on the other hand, only reduced the Ca^{2+} increase by about 50% at this concentration, the highest tested [8]. In mouse sensory neurons in vitro, it has been shown that lidocaine (2 mM) does not completely prevent capsaicin-induced increases in intracellular Ca^{2+}, nor does it seem to prevent acute desensitization of capsaicin responses [2]. The results of our study suggest that bupivacaine may be a more potent inhibitor of TRPV1 than other local anesthetics, but its maximal efficacy seems to be limited. Our finding that tetracaine enhanced capsaicin-induced currents is novel and suggests that it may be the local anesthetic of choice for blocking the undesirable initial pain of capsaicin application while maintaining and perhaps even enhancing the therapeutic desensitizing effect of capsaicin.

The kinetics of the interaction between local anesthetics and the TRPV1 channel appear rapid. In all of our experiments, local anesthetics were not preapplied to the cell, instead administered at the same time as capsaicin. The effects of the local anesthetics developed rapidly and appeared maximal by the end of the 2.5 s application. The secondary increase in the capsaicin-induced current which we observed after removal of bupivacaine and capsaicin also suggests a rapid dissociation of bupivacaine from the TRPV1 channel, leaving a nearly normal capsaicin current magnitude within a few seconds of washout of bupivacaine. The absence of a biphasic effect during persistent application of bupivacaine after removal of capsaicin confirmed that

the secondary increase we observed was likely due to rapid washout of bupivacaine, leaving the residual effect of capsaicin.

In summary, we have shown that local anesthetics may have moderate inhibitory effects (bupivacaine) or even a stimulating effect (tetracaine) on the initial ion influx induced by capsaicin. These findings may have relevance for the clinical use of local anesthetics along with capsaicin for the treatment of chronic pain states.

Acknowledgements

Supported by an NIH K08 Award (GM64672-01) and the Department of Anesthesiology, the University of Wisconsin, Madison WI. The authors thank Craig V. Levenick for expert technical assistance.

References

1. Avelino A, Cruz F, Coimbra A. Lidocaine prevents noxious excitation of bladder afferents induced by intravesical capsaicin without interfering with the ensuing sensory desensitization: an experimental study in the rat. J Urol 1998;159:567–570. [PubMed: 9649293]
2. Bonnington JK, McNaughton PA. Signaling pathways involved in the sensitization of mouse nociceptive neurones by nerve growth factor. J Physiol 2003;551.2:433–446. [PubMed: 12815188]
3. Craft RM, Porreca F. Tetracaine attenuates irritancy without attenuating desensitization produced by intravesical resiniferatoxin in the rat. Pain 1994;57:351–359. [PubMed: 7936713]
4. Eun SY, Jung SJ, Park YK, Kwak J, Kim SJ, Kim J. Effects of capsaicin on Ca^{2+} release from the intracellular Ca^{2+} stores in the dorsal root ganglion cells of adults rats. Biochem Biophys Res Commun 2001;285:1114–1120. [PubMed: 11478769]
5. Gold MS, Reichling DB, Hampl KF, Drasner K, Levine JD. Lidocaine toxicity in primary afferent neurons from the rat. J Pharmacol Exp Ther 1998;285:413–421. [PubMed: 9580578]
6. Gover TD, Kao JPY, Weinreich D. Calcium signaling in single peripheral sensory nerve terminals. J Neurosci 2003;23:4793–4797. [PubMed: 12832498]
7. Greffrath W, Kirschstein T, Nawrath H, Treede RD. Changes in cytosolic calcium in response to noxious heat and their relationship to vanilloid receptors in rat dorsal root ganglion neurons. Neuroscience 2001;104:539–550. [PubMed: 11377853]
8. Hirota K, Smart D, Lambert DG. The effects of local and intravenous anesthetics on recombinant rat TRPV1 vanilloid receptors. Anesth Analg 2003;96:1656–1660. [PubMed: 12760991]
9. Koplas PA, Rosenberg RL, Oxford GS. The role of calcium in the desensitization of capsaicin responses in rat dorsal root ganglion neurons. J Neurosci 1997;17:3525–3537. [PubMed: 9133377]
10. Liu M, Liu MC, Magoulas C, Priestley JV, Willmott NJ. Versatile regulation of cytosolic Ca^{2+} by vanilloid receptor I in rat dorsal root ganglion neurons. J Biol Chem 2003;278:5462–5472. [PubMed: 12454015]
11. Németh J, Helyes Z, Oroszi G, Jkab B, Pintér E, Szilvássy Z, Szolcsányi. Role of voltage-gated cation channels and axon reflexes in the release of sensory neuropeptides by capsaicin from isolated rat trachea. Eur J Pharmacol 2003;458:313–318. [PubMed: 12504788]
12. Vlachovà V, Vitàskovà Z, Vyklicky L, Orkand RK. Procaine excites nociceptors in cultures from dorsal root ganglion of the rat. Neurosci Lett 1999;263:49–52. [PubMed: 10218908]

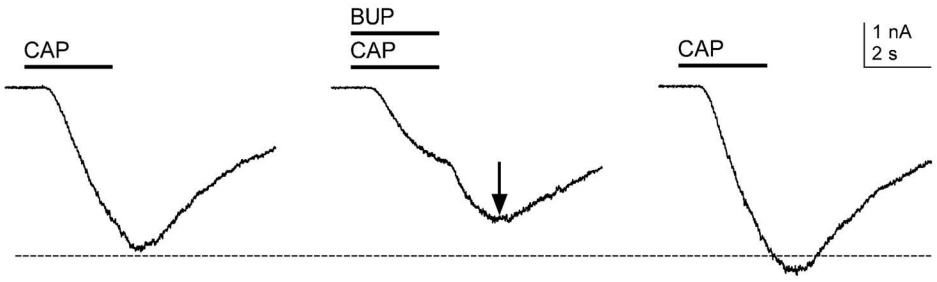

Fig 1.

Capsaicin-induced currents in the absence and presence of 1 mM bupivacaine. The left panel shows the control capsaicin-induced current, the middle panel the capsaicin-induced current in the presence of bupivacaine, and the right panel the recovery capsaicin-induced current (after bupivacaine). The bars at the top of each trace indicate the time of perfusion with capsaicin and bupivacaine. CAP, 1 μM capsaicin; BUP, 1 mM bupivacaine. The arrow in the middle panel denotes the peak of the secondary increase in the capsaicin current after washout of capsaicin and bupivacaine. The traces were recorded approximately 1 min apart.

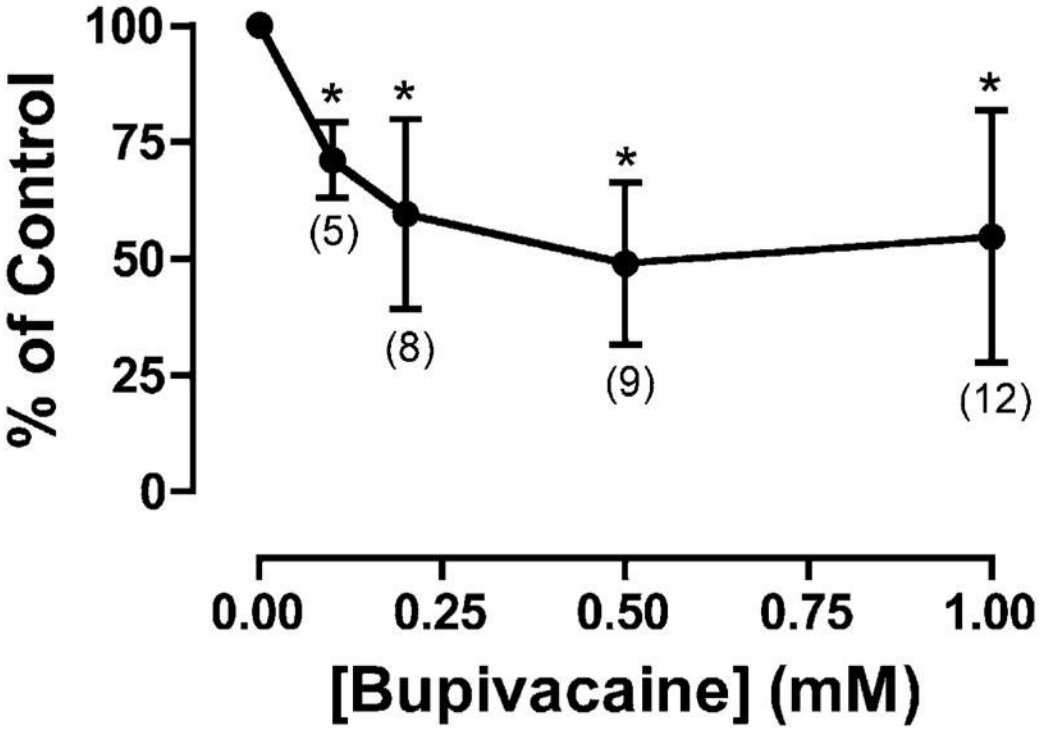

Fig 2.

The concentration dependence of the reduction of the capsaicin-induced current by bupivacaine. The points and error bars represent means ± standard deviations. Only one concentration of bupivacaine was tested in each cell. The number of cells tested at each concentration is shown in parentheses. The asterisks (*) indicate significant decreases in the magnitude of the capsaicin currents compared to the corresponding control peak current, $p < 0.05$.

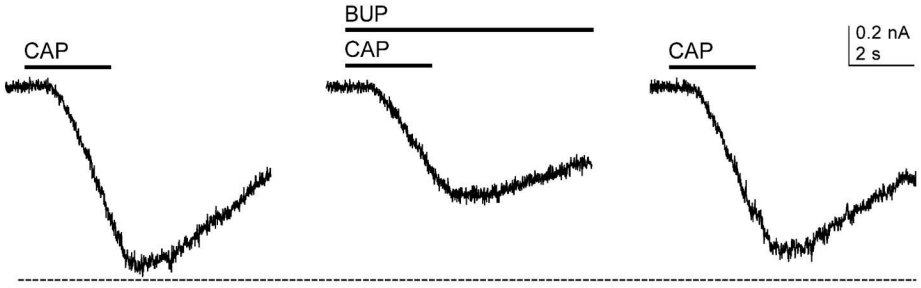

Fig 3.

Absence of a secondary increase in the capsaicin current when bupivacaine is present after washout of capsaicin. The left panel shows the control capsaicin-induced current, the middle panel the capsaicin-induced current in the presence of bupivacaine, and the right panel the recovery capsaicin-induced current (after bupivacaine). The bars at the top of each trace indicate the time of perfusion with capsaicin and bupivacaine. CAP, 1 μM capsaicin; BUP, 1 mM bupivacaine. The traces were recorded approximately 1 min apart.

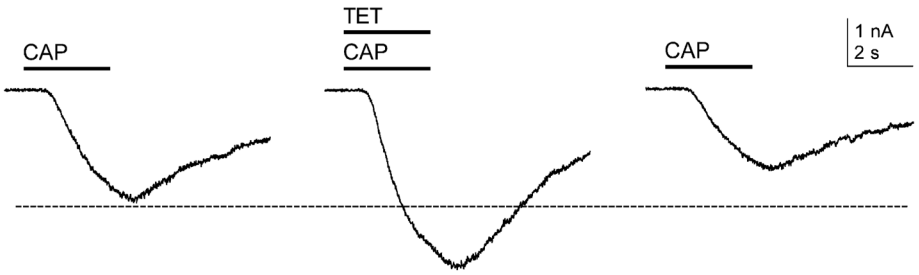

Fig 4.

Capsaicin-induced currents in the absence and presence of 1 mM tetracaine. The left panel shows the control capsaicin-induced current, the middle panel the capsaicin-induced current in the presence of tetracaine, and the right panel the recovery capsaicin-induced current (after tetracaine). The bars at the top of each trace indicate the time of perfusion with capsaicin and tetracaine. CAP, 1 μM capsaicin; TET, 1 mM tetracaine. The traces were recorded approximately 1 min apart.

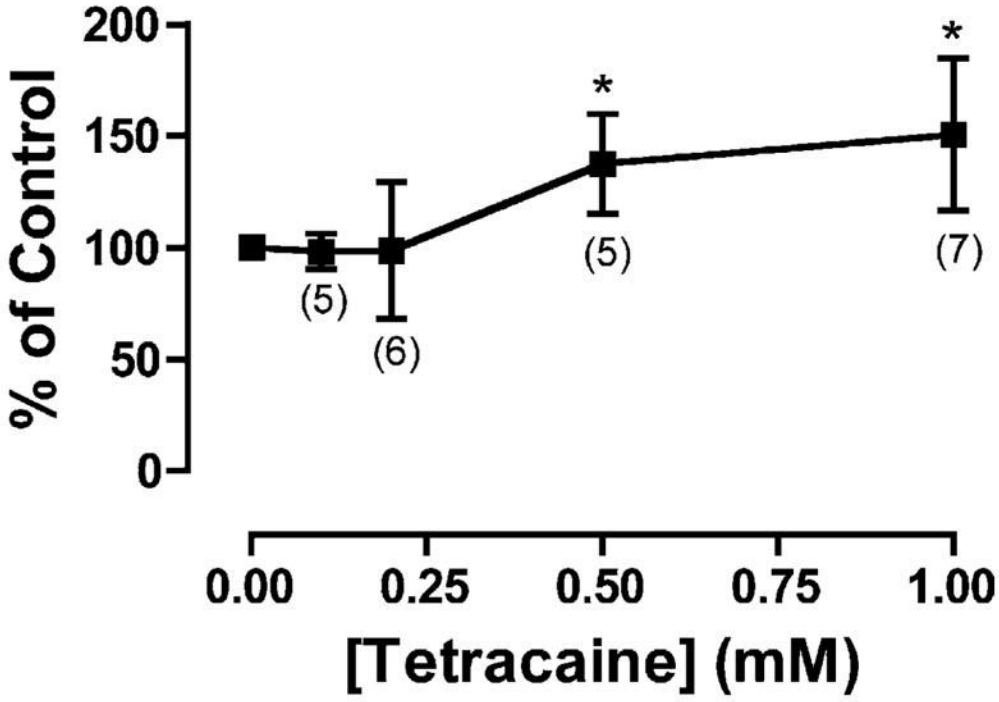

Fig 5.

The concentration dependence of the enhancement of the capsaicin-induced current by tetracaine. The points and error bars represent means ± standard deviations. Only one concentration of tetracaine was tested in each cell. The number of cells tested at each concentration is shown in parentheses. The asterisks (*) indicate significant increases in the magnitude of the capsaicin currents compared to the corresponding control peak current, $p < 0.05$.