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Influence of Voltage-sensitive Ca^{++} Channel Drugs on Bupivacaine Infiltration Anesthesia in Mice

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Background: **Local anesthesia has been traditionally associated with blockade of voltage-sensitive sodium (Na^+) channels. Yet *in vitro* evidence indicates that local anesthetic mechanisms are more complex than previously understood. For example, local anesthetics bind and allosterically modify 1,4-dihydropyridine-sensitive Ca^{++} channels and can reduce Ca^{++} influx in tissues. The current study examines the influence of voltage-sensitive Ca^{++} channels in bupivacaine infiltration anesthesia.**

Methods: **Baseline tail-flick latencies to radiant heat nociception were obtained before subcutaneous infiltration of bupivacaine and Ca^{++}-modulating drugs in the tails of mice. No musculature is contained in the tail that could result in motor block. The magnitude of infiltration anesthesia over time, as well as the potency of bupivacaine alone or in the presence of Ca^{++}-modulating drug, was assessed by obtaining test latencies.**

Results: **The 1,4-dihydropyridine L-type Ca^{++} channel agonist *S*(−)-BayK-8644 reduced the duration of action and potency of bupivacaine anesthesia. In opposite fashion, nifedipine and nicardipine increased the effects of bupivacaine. Neither nifedipine nor nicardipine alone elicited anesthesia. Alternatively, the phenylalkylamine L-type blocker verapamil elicited concentration-dependent anesthesia. Other Ca^{++} channel subtype blockers were investigated as well. The N-, T-, P-, and Q-type channel blockers, ω-conotoxin GVIA, flunarizine, ω-agatoxin IVA, and ω-conotoxin MVIIC, respectively, were unable to modify bupivacaine anesthesia.**

Conclusions: **These results indicate that heat nociception stimulates Ca^{++} influx through L-type channels on nociceptors in skin. Although other voltage-sensitive Ca^{++} channels may be located on skin nociceptors, only the L-type channel drugs affected bupivacaine in the radiant heat test.**

VOLTAGE-sensitive Ca^{++} channels are known to open in response to membrane depolarization resulting from the generation of nerve impulses.[1] The influx of Ca^{++} is essential for maintaining normal sensory processing, the transmission of nerve impulses, and synaptic transmission. For example, Ca^{++}-sensitive K^+ channels regulate sensory neuronal excitability.[2] Excitability is diminished when Ca^{++} influx from action potentials opens more Ca^{++}-sensitive K^+ channels, leading to membrane hyperpolarization.[3] Furthermore, activation of Ca^{++}-sensitive kinases and phosphatases can affect the phosphorylation state and function of voltage-gated ion channels. For example, protein kinase A and protein kinase C phosphorylate voltage-gated Na^+ channels, whereas calcineurin is thought to dephosphorylate these channels.[4,5]

It is widely accepted that local anesthetics reversibly prevent the conduction of electrical impulses in nerves by blocking voltage-gated Na^+ channels.[6] Yet the properties of local anesthetics appear to be more complex than previously understood. Ligand binding assays on 1,4-dihydroypridine–sensitive Ca^{++} channels have revealed the existence of an allosteric binding site for local anesthetics.[7,8] Functionally, local anesthetics can directly depress Ca^{++} entry into tissue.[9,10] Thus, anesthesia may also reflect the actions of local anesthetics in altering Ca^{++} influx and release from intracellular pools. This study was conducted to test the hypothesis that voltage-sensitive Ca^{++} channels play an important role in regulating the anesthetic properties of bupivacaine in mice.

In the current study, mice were tested for infiltration anesthesia in the radiant-heat tail-flick test after Ca^{++} modulating drugs were coadministered with bupivacaine in the tail. Our results revealed that only the 1,4-dihydropyridine–sensitive L-type Ca^{++} channel drugs were able to modulate the anesthetic effects of bupivacaine.

Materials and Methods

Methods of Handling Mice

Male Institute for Cancer Research (ICR) mice (Harlan Laboratories, Indianapolis, IN) weighing 27.0 ± 0.4 g were housed six to a cage in animal-care quarters maintained at 22 ± 2°C on a 12-h light–dark cycle. Food and water were available *ad libitum*. The mice were brought to a test room (22 ± 2°C, 12-h light–dark cycle), marked for identification, and allowed 16 h to recover from transport and handling. The Institutional Animal Care and Use Committee at the Medical College of Virginia Campus of Virginia Commonwealth University (Richmond, VA) approved all procedures in the study.

The Tail-flick Test

The tail-flick test used to assess for infiltration anesthesia was developed by D'Amour and Smith[11] and modified by Dewey *et al.*[12] The tail-flick device is designed to focus a light beam onto the site infiltrated with local anesthetic, without exposing noninjected tissue to the

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noxious radiant-heat stimulus. The intensity was adjusted to yield baseline latencies of between 2 and 4 s. A single baseline latency was obtained approximately 15 min before the mice were injected with drug. A 10-s cutoff was used to prevent any potential tissue damage. In experiments testing for potential underlying infiltration anesthesia that was not observed with the higher heat stimulus, the intensity was reduced to yield baseline latencies of approximately 6 s. A 12-s cutoff was used in these experiments.

Subcutaneous Infiltration of Bupivacaine

Before infiltration of vehicle or drug, baseline tail-flick latencies were obtained by exposing the tail to noxious radiant heat at the site to be infiltrated with drug. The baseline average over the entire study was 3.1 ± 0.1 s. The mice were then restrained to allow unencumbered access to the tail. A 26-gauge ⅝-inch needle was inserted approximately 1.0–1.3 cm longitudinally under the skin in the subcutaneous space in the dorsal aspect of the tail. The needle was attached to a 1-ml syringe (Becton Dickinson, Franklin Lakes, NJ) prefilled with drug solution. A 100-μl volume of drug solution was infiltrated, and the needle was left in place for 5 s to prevent leakage of injectate from the site after removing the needle. During injection, the solution dispersed around the circumference of the tail in a rostral-caudal direction of approximately 2 to 3 cm. A slight blanching of the skin that reversed within 1–2 min indicated the extent of the spread of solution. A nontoxic marking pen was used to mark the most rostral spread of solution. Tail-flick testing was conducted below the area marked on the tail. These methods are detailed in a previous report from this laboratory.[13]

Experimental Design

All test drugs were solubilized and then mixed with commercial bupivacaine for coinfiltration of the tail. The time course for Ca^{++}-modulating drugs to affect bupivacaine anesthesia was examined. These studies comprised the following treatment groups: vehicle + saline, test drug + saline, vehicle + bupivacaine, and test drug + bupivacaine. Baseline tail-flick latencies were obtained, after which test latencies were collected at predetermined times after drug administration. The ordinate was expressed as tail-flick latency (seconds), while the abscissa was expressed as time (minutes). The influence of increasing concentrations of Ca^{++}-modulating drug on the ED_{50} dose of bupivacaine was also examined. Both baseline and test tail-flick latencies from each animal were converted into the percentage of maximum possible effect (%MPE) according to the method of Harris and Pierson,[14] which was calculated as follows:

$$\%MPE = [(test - baseline)/(10 - baseline)] \times 100.$$

The ordinate was %MPE, while the abscissa was expressed as the concentration of Ca^{++}-modulating drug. For active Ca^{++}-modulating drugs, complete bupivacaine dose–response curves were generated in the absence and presence of Ca^{++}-modulating drug for calculation of ED_{50} and potency ratio values. The ordinate was %MPE, while the abscissa was expressed as the percentage of bupivacaine dose.

Statistical Analyses

The tail-flick latency (seconds) values were analyzed with one-factor analysis of variance for experiments in which different concentrations of Ca^{++} channel drug were tested with an ED_{50} dose of bupivacaine. A statistically significant F value led to *post hoc* comparisons using the Tukey test. Tail-flick latency (seconds) values were analyzed with two-factor repeated measures analysis of variance for experiments that examined the time course of Ca^{++}-modulating drugs to affect bupivacaine anesthesia. A significant F value for the drug treatment × time interaction led to *post hoc* analysis using the Tukey test. The %MPE values for dose–response curves were analyzed in the following manner. ED_{50} values were calculated using least squares linear regression analysis of %MPE values followed by calculation of 95% confidence limits according to the method of Bliss.[15] Dose–response curves were considered significantly different if the 95% confidence limits did not overlap. Tests for parallelism were conducted before calculation of potency ratio values and 95% confidence limits by the method of Colquhoun.[16] A potency ratio value greater than 1, with a lower 95% confidence limit

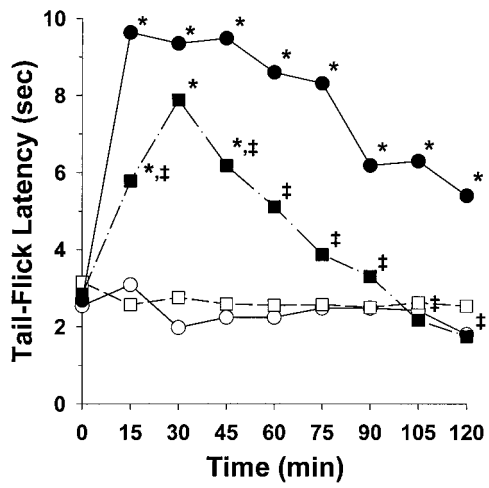

Fig. 1. *S*(−)-BayK-8644 reduced the magnitude and duration of bupivacaine local anesthesia. Baseline tail-flick latencies to radiant-heat nociception were obtained before injecting 0.1 ml of vehicle or *S*(−)-BayK-8644 (35 μM) mixed with saline or 0.43% bupivacaine. At the indicated times, the animals were tested for local anesthesia. The groups consisted of vehicle + saline (open circle, solid line), *S*(−)-BayK-8644 + saline (open square, dashed line), vehicle + bupivacaine (filled circle, solid line), and *S*(−)-BayK-8644 + bupivacaine (filled square, dash-dotted line). **P* < 0.05 compared with baseline latency; ‡*P* < 0.05 compared with corresponding vehicle + bupivacaine time point.

greater than 1, was considered a significant difference in potency.

Drugs

Bupivacaine HCl in a stock concentration of 0.75% was purchased from the Medical College of Virginia Hospitals Pharmacy (AstraZeneca, Westborough, MA). When necessary, dilutions of bupivacaine were made with sterile isotonic saline to achieve the desired final percent solution (Baxter Healthcare Corp., Deerfield, IL). *S*(−)-BayK-8644 (Research Biochemicals International, Natick, MA), nifedipine, nicardipine (Sigma Chemical Co., St. Louis, MO), and flunarizine (Calbiochem, San Diego, CA) were dissolved in a DMSO–emulphor mixture and diluted to a final vehicle concentration of 5% DMSO, 10% emulphor, and 85% bupivacaine solution. The vehicle control consisted of 5% DMSO, 10% emulphor, and 85% isotonic saline. The water-soluble drugs ω-conotoxin GVIA (Research Biochemicals International), ω-agatoxin IVA, and ω-conotoxin MVIIC (Calbiochem, San Diego, CA) were dissolved in saline and then added to the bupivacaine solution. The vehicle control consisted of isotonic saline.

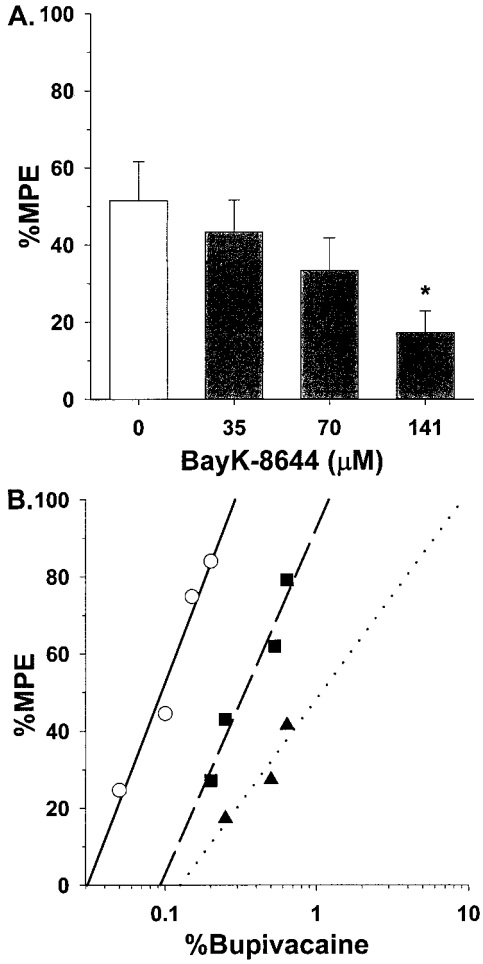

Fig. 2. (*A*) Dose-dependent inhibition of bupivacaine with *S*(−)-BayK-8644. Baseline tail-flick latencies were obtained before injecting 0.1 ml of vehicle or *S*(−)-BayK-8644 (35 and 141 μM) mixed with 0.12% bupivacaine. The mice were tested for local anesthesia 30 min later. **P* < 0.05 compared with the vehicle + bupivacaine control. (*B*) *S*(−)-BayK-8644 reduced the potency of bupivacaine anesthesia. Baseline tail-flick latencies were obtained before injecting mice with 0.1 ml bupivacaine mixed with vehicle (open circle, solid line) or *S*(−)-BayK-8644 (35 μM [filled square, dashed line] or 141 μM [filled triangle, dotted line]). The mice were tested for local anesthesia 30 min later. Each dose–response curve represents 18–24 mice. %MPE = percentage of maximum possible effect.

Table 1. Influence of the Dihydropyridine Ca^{++} Channel Opener *S*(−)-BayK-8644 on Bupivacaine Local Anesthesia

Treatment	ED_{50} % Bupivacaine (95% CL)		Potency Ratio (95% CL)
Vehicle	0.10 (0.08–0.12)		—
S(−)-BayK-8644 (μM)			
35	0.33 (0.23–0.50)*	vs. Veh	3.6 (2.2–6.2)*
141	1.08 (0.85–1.36)*	vs. Veh	8.1 (6.9–16.6)*

Baseline latencies were obtained from mice by exposing their tails to noxious radiant heat. A 100-μl volume of drug mix was then infiltrated subcutaneously in the tail at the site of the radiant heat exposure. The drug mix consisted of vehicle or *S*(−)-BayK-8644 mixed with different percent bupivacaine solutions for construction of bupivacaine dose–response curves. Test latencies were obtained 30 min later.

* Significantly different from vehicle group.

CL = confidence limit.

Results

Role of L-type Voltage-sensitive Ca^{++} Channels in Bupivacaine Infiltration Anesthesia

Experiments were designed to determine the role of 1,4-dihydropyridine–sensitive Ca^{++} channels (L-type) in the expression of bupivacaine infiltration anesthesia. Vehicle or the 1,4-dihydropyridine Ca^{++} channel opener *S*(−)-BayK-8644[17] was coadministered with bupivacaine. As shown in figure 1, bupivacaine injected alone elicited anesthesia that was still present 2 h later. *S*(−)-BayK-8644 injected alone did not affect the tail-flick latency over the 2-h test period compared with mice injected with vehicle. However, coinfiltration of *S*(−)-BayK-8644 with bupivacaine significantly reduced the magnitude and duration of anesthesia ($F_{3,24} = 6.23$; $P < 0.001$). In addition, *S*(−)-BayK-8644 blocked the effect of an ED_{50} dose of bupivacaine in a concentration-dependent manner (fig. 2A) and significantly reduced the potency of bupivacaine (fig. 2B, table 1).

Alternatively, blockade of 1,4-dihydropyridine–sensitive Ca^{++} channels with nifedipine (fig. 3A) or nicardipine (fig. 3B) enhanced the effects of bupivacaine. As shown in figure 3A, nifedipine alone did not significantly affect tail-flick latencies compared with vehicle-injected mice during the 120-min test period. However, nifedipine significantly enhanced the duration of bupivacaine infiltration anesthesia ($F_{3,24} = 11.89$; $P < 0.001$). Nifed-

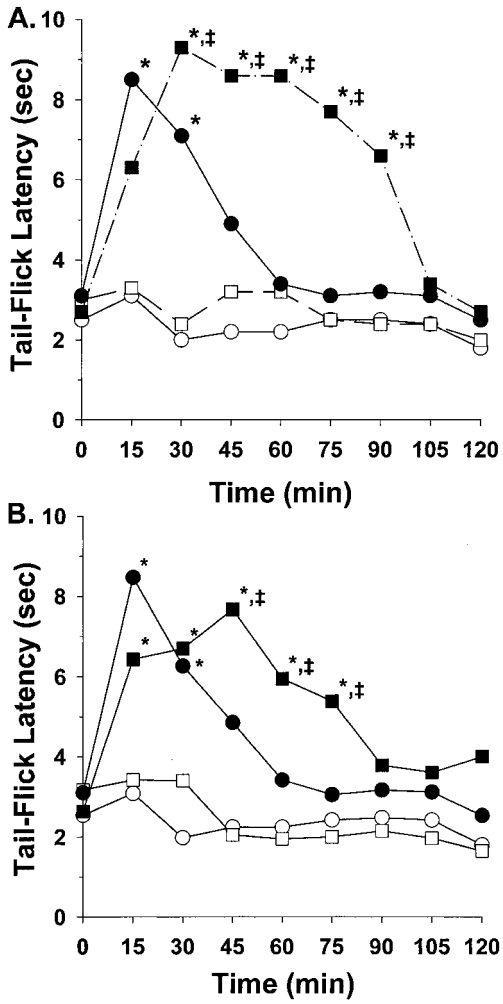

Fig. 3. (*A*) Nifedipine increased the duration of bupivacaine anesthesia. Baseline tail-flick latencies were obtained before injecting 0.1 ml of vehicle or nifedipine (12 mM) mixed with saline or 0.12% bupivacaine. At the indicated times, the animals were tested for local anesthesia. The groups consisted of vehicle + saline (open circle, solid line), nifedipine + saline (open square, dashed line), vehicle + bupivacaine (filled circle, solid line), and nifedipine + bupivacaine (filled square, dash-dotted line). *P < 0.05 compared with baseline latency; ‡P < 0.05 compared with corresponding vehicle + bupivacaine time point. (*B*) Nicardipine increased the duration of bupivacaine anesthesia. Baseline tail-flick latencies were obtained before injecting 0.1 ml of vehicle or nicardipine (3875 μM) mixed with saline or 0.12% bupivacaine. At the indicated times, the animals were tested for local anesthesia. The groups consisted of vehicle + saline (open circle, solid line), nicardipine + saline (open square, dashed line), vehicle + bupivacaine (filled circle, solid line), and nicardipine + bupivacaine (filled square, dash-dotted line). *P < 0.05 compared with baseline latency; ‡P < 0.05 compared with corresponding vehicle + bupivacaine time point.

ipine increased the anesthetic effects of an ED_{50} dose of bupivacaine (fig. 4A) and significantly increased the potency of bupivacaine (fig. 4B, table 2). Because nifedipine is a vasodilator, the concern was raised that the enhancement in anesthesia reflected the systemic effects of bupivacaine absorbed into circulation. Mice injected with 0.12% bupivacaine + vehicle and 0.12% bupivacaine + nifedipine (28.8 μM) in the tail were tested with the hot-plate paw-withdrawal assay (56°C). Nifedipine did not affect the paw-withdrawal latencies (*i.e.*, 22% *vs.* 17% MPE, respectively). In addition, anesthesia was absent in the tail-flick test after conventional subcutaneous administration of bupivacaine + vehicle and bupivacaine + nifedipine in the back of the neck (*i.e.*, 8.9% *vs.* 3.2% MPE, respectively). The effects of nifedipine were confirmed with experiments in which the 1,4-dihydropyridine nicardipine was tested. Like nifedipine, nicardipine alone elicited no anesthesia but enhanced the duration of bupivacaine anesthesia ($F_{3,24}$ = 4.10; P < 0.001; fig. 3B). Nicardipine also increased the anesthetic effects of an ED_{50} dose of bupivacaine (fig. 5A) and significantly increased the potency of bupivacaine (fig. 5B, table 2).

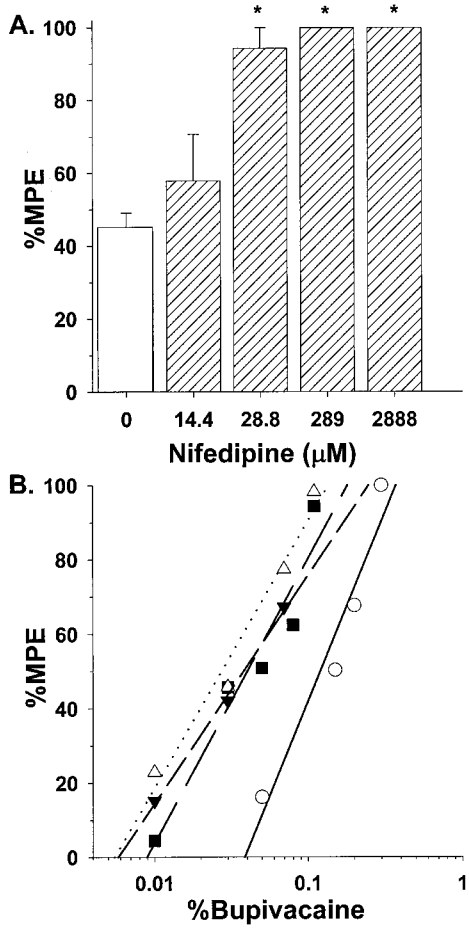

Fig. 4. (*A*) Dose-dependent enhancement of bupivacaine with nifedipine. Baseline tail-flick latencies were obtained before injecting 0.1 ml of vehicle or nifedipine (14.4–2,880 μM) mixed with 0.12% bupivacaine. The mice were tested for local anesthesia 30 min later. *P < 0.05 compared with the vehicle + bupivacaine control. (*B*) Nifedipine increased the potency of bupivacaine. Baseline tail-flick latencies were obtained before injecting mice with 0.1 ml bupivacine mixed with vehicle (open circle, solid line) or nifedipine (28.8 μM [filled square, long-dash line], 289 μM [open triangle, dotted line], 2,888 μM [filled triangle, short-dash line]). Each dose–response curve represents 24–30 mice. %MPE = percentage of maximum possible effect.

Table 2. Influence of Dihydropyridine-sensitive Ca^{++} Channel Blockade on Bupivacaine Local Anesthesia

Treatment	ED_{50} % Bupivacaine (95% CL)	Potency Ratio (95% CL)
Vehicle	0.12 (0.10–0.16)	—
Nifedipine (μM)		
29	0.04 (0.03–0.05)*	*vs.* Veh 3.0 (2.0–4.5)*
289	0.03 (0.02–0.03)*	*vs.* Veh 4.7 (2.9–7.4)*
2,888	0.04 (0.02–0.06)*	*vs.* Veh 3.5 (1.8–6.1)*
Nicardipine (μM)		
194	0.10 (0.08–0.14)	*vs.* Veh 1.2 (0.8–1.8)
1,938	0.04 (0.03–0.06)*	*vs.* Veh 2.7 (1.6–5.2)*
3,875	0.03 (0.02–0.05)*	*vs.* Veh 3.5 (2.1–6.1)*

Baseline latencies were obtained from mice by exposing their tails to noxious radiant heat. A 100-μl volume of drug mix was then infiltrated subcutaneously in the tail at the site of the radiant heat exposure. The drug mix consisted of vehicle, nifedipine, or nicardipine mixed with different percent bupivacaine solutions for construction of bupivacaine dose–response curves. Test latencies were obtained 30 min later.

* Significantly different from vehicle group.

CL = confidence limit.

The phenylalkylamine L-type blocker verapamil was also tested with the intention of validating the results with the 1,4-dyhydropyridines. Like the preceding experiments, the mice were tested with a noxious heat stimulus that yielded baseline latencies of approximately 3 s. However, verapamil infiltration elicited an anesthetic effect similar to that reported in humans.[18] Figure 6A reveals that the duration of anesthesia increased with verapamil dose. In addition, the calculated ED_{50} value of verapamil from the dose–response curve was 3.2 mM (95% confidence limit, 2.8–3.7; fig. 6B). This led us to speculate that nifedipine and nicardipine might possess some anesthetic properties that were obscured by the high-intensity stimulus experiments conducted in figures 3–5. Thus, for these experiments, the light intensity was reduced to yield baseline latencies of approximately 6 s. Yet even during these very mild noxious heat conditions, nifedipine and nicardipine were inactive in the tail-flick test (figs. 7A and B). These results indicate that verapamil is unique among Ca^{++} channel blockers in eliciting anesthesia.

Role of N-, T-, P-, and Q-type Ca^{++} Channels in Bupivacaine Infiltration Anesthesia

Other voltage-sensitive Ca^{++} channels were examined for their role in modulating bupivacaine infiltration anesthesia. Of the Ca^{++} channel blockers to be mentioned, none elicited anesthesia when injected alone, despite testing a 100-fold range of concentrations. In addition, none of these Ca^{++} channel blockers altered the anesthetic effects of an ED_{50} dose of bupivacaine. The N- and T-type blockers ω-conotoxin GVIA and flunarizine, respectively, failed to enhance or block bupivacaine (figs. 8A and B). In addition, the P- and Q-type blocker ω-agatoxin IVA ω-conotoxin MVIIC, respectively, failed to enhance or block bupivacaine (figs. 9A and B).

Discussion

Infiltration Anesthesia by Ca^{++} Channel Blockers

Both electrophysiologic and behavioral experiments have shown that blockers of L-, N-, and P-type Ca^{++} channels on the nerve terminals of A-δ–C fibers innervating the dorsal spinal horn block nociceptive input.[19–22] Yet less is known about the identity of Ca^{++} channels present on nociceptors in the organs and skin innervated by these sensory neurons. Nociceptors in the cornea appear to possess L-type Ca^{++} channels. Application of diltiazem on the cornea elicited concentration-dependent depression of cold fiber discharge activity, without affecting mechanoreceptor afferents.[23] Studies on nociceptors in rat skin flaps indicate that L- and N-type channels, but not P- or Q-type, block KCl-stimulated calcitonin gene-related peptide release from small-

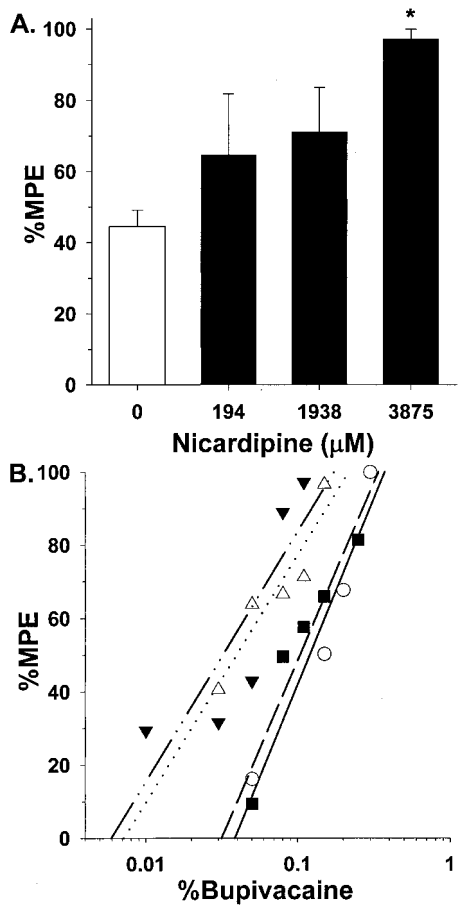

Fig. 5. (*A*) Dose-dependent enhancement of bupivacaine with nicardipine. Baseline tail-flick latencies were obtained before injecting 0.1 ml vehicle or nicardipine (194–3,875 μM) mixed with 0.12% bupivacaine. The mice were tested for local anesthesia 30 min later. *$P < 0.05$ compared with the vehicle + bupivacaine control. (*B*) Nicardipine increased the potency of bupivacaine. Baseline tail-flick latencies were obtained before injecting mice with 0.1 ml bupivacaine mixed with vehicle (open circle, solid line) or nicardipine (194 μM [filled square, dash line], 1,938 μM [filled triangle, dotted line], 3,875 μM [open triangle, dash-dotted line]). The mice were tested for local anesthesia 30 min later. Each dose–response curve represents 24–30 mice. %MPE = percentage of maximum possible effect.

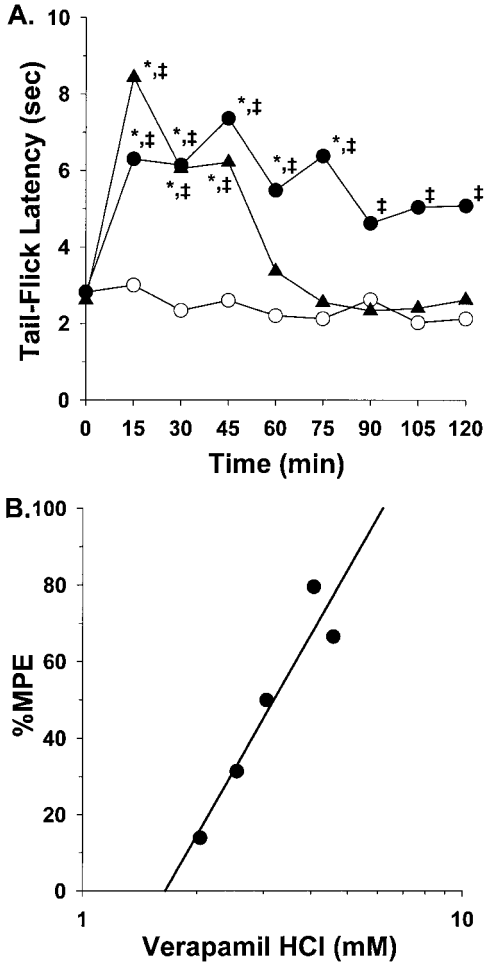

Fig. 6. (*A*) Verapamil elicited dose-dependent increases in the duration of local anesthesia. Baseline tail-flick latencies to radiant-heat nociception were obtained before injecting vehicle or verapamil. At the indicated times, the animals were tested for local anesthesia. The groups consisted of vehicle (open circle, solid line) or verapamil (filled triangle, 4.1 mM; filled circle, 16.0 mM). *$P < 0.05$ compared with baseline latency; ‡$P < 0.05$ compared with corresponding vehicle time point. (*B*) Verapamil elicited dose-dependent local anesthesia. Baseline tail-flick latencies were obtained before injecting mice with verapamil (filled circle, solid line). The mice were tested for local anesthesia 15 min later. The dose–response curve represents 30 mice. %MPE = percentage of maximum possible effect.

diameter unmylenated afferent neurons.[24] Of course, other high- and low-voltage–activated Ca^{++} channels (*e.g.*, T-, P-, Q types, and others) may be present on nociceptors and participate in transmitting chemical, mechanical, or other forms of nociception.

This study focused on the role of voltage-sensitive Ca^{++} channels in sensory transduction, which is the process by which nociceptors convert noxious radiant heat into impulses that ultimately lead to behavioral responses. In humans, intradermal injection of 0.5 ml of 388 μM nicardipine elicited a mild but statistically significant increase (*i.e.*, 1.45 s) in the pain threshold to noxious radiant heat.[25] Interestingly, in mice some concentrations of nifedipine and nicardipine caused nonsignificant increases in low-intensity tail-flick latencies of 1.4 and 2.4 s, respectively. In this assay, a 1.4–2.4-s increase in tail-flick latencies is very mild considering that bupivacaine can increase latencies 7 to 8 s above the baseline during high-intensity conditions. Alternatively, verapamil (2.0–4.6 mM) elicited anesthesia in mice, which was consistent with local anesthesia in humans administered 5-mM concentrations.[18] The 1,4-dihydropyridines are highly selective inhibitors of voltage-sensitive Ca^{++} channels. However, verapamil can also block voltage-activated fast Na^{+} currents and inhibit the amplitude of compound action potentials in sciatic nerves isolated from rats.[26] Thus, the anesthetic effects of verapamil

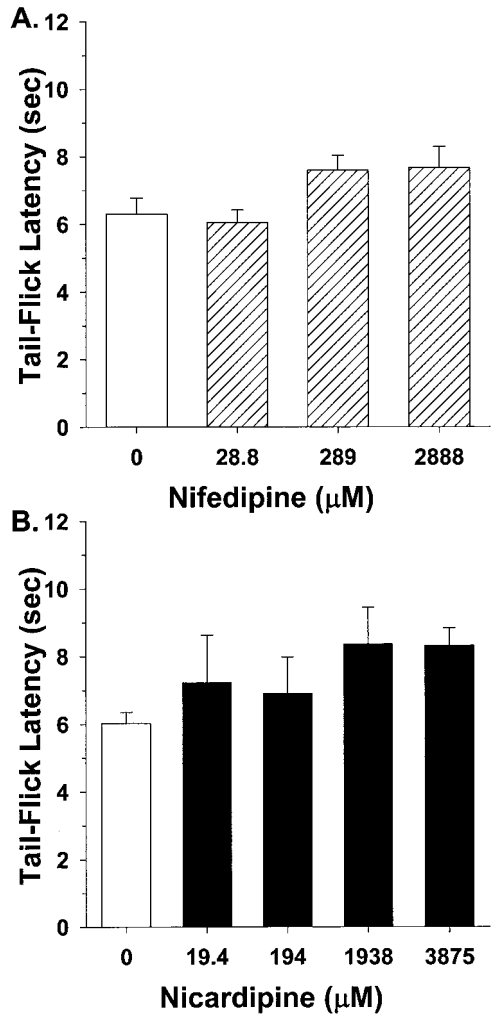

Fig. 7 (*A*) Assessment of nifedipine local anesthesia using a low-intensity tail-flick stimulus. The radiant-heat intensity was reduced to yield baseline tail-flick latencies of approximately 6 s. This allowed us to assess for any mild local anesthesia that was not observed with the high-intensity stimulus. Mice were injected with vehicle or nifedipine (28.8–2,888 μM) and assessed for local anesthesia 30 min later. (*B*) Assessment of nicardipine local anesthesia using a low-intensity tail-flick stimulus. The radiant heat intensity was reduced to yield baseline tail-flick latencies of approximately 6 s. Mice were injected with vehicle or nicardipine (19.4–3,875 μM) and assessed for local anesthesia 30 min later.

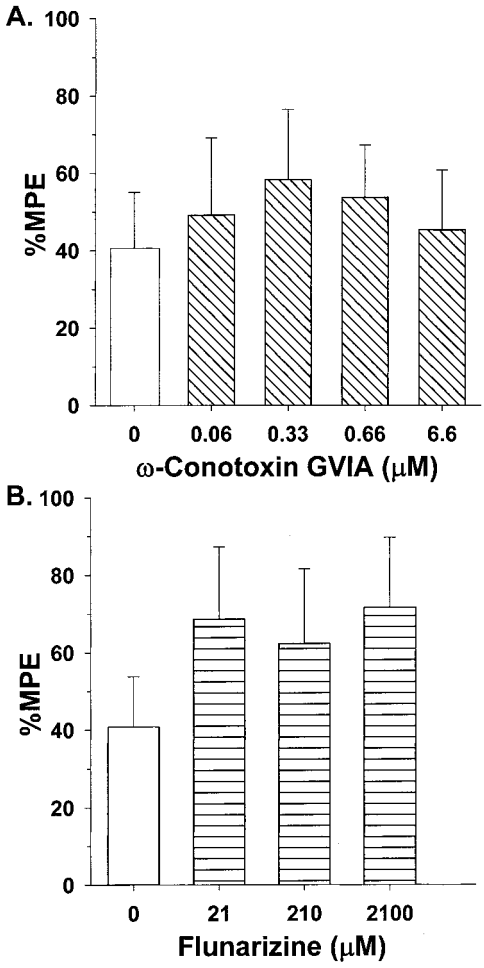

Fig. 8. (*A*) ω-Conotoxin GIVA did not affect bupivacaine anesthesia. Baseline tail-flick latencies were obtained before injecting vehicle or ω-conotoxin GIVA (0.06–6.6 μM) mixed with 0.12% bupivacaine. Local anesthesia was tested 30 min later. (*B*) Flunarizine did not affect bupivacaine anesthesia. Baseline tail-flick latencies were obtained before injecting vehicle or flunarazine (21–2,100 μM) mixed with 0.12% bupivacaine. The mice were tested for local anesthesia 30 min later. %MPE = percentage of maximum possible effect.

could reflect the blockade of both Na^+ and Ca^{++} channels.

Role of L-type Voltage-sensitive Ca^{++} Channels in Bupivacaine Infiltration Anesthesia

Our results indicate that Ca^{++} influx through the α-1C voltage-dependent pore of L-type channels plays an important role in the anesthetic effects of bupivacaine. Activation of Ca^{++} channels with *S*(−)-BayK-8644 reduced the potency and duration of bupivacaine anesthesia, whereas blockade by nifedipine or nicardipine increased the effects of bupivacaine. Iwasaki *et al.*[27] reported that several L-type Ca^{++} channel blockers, including nicardipine, enhanced lidocaine anesthesia in mice. As in our study, the mice were tested in the tail-flick assay after subcutaneous infiltration of the tail with lidocaine alone or mixed with nicardipine.

The evidence clearly demonstrates that L-type Ca^{++}-modulating drugs interact pharmacologically with bupivacaine. Each drug could be acting selectively at their respective binding sites to affect Na^+ and Ca^{++} influx. However, the interaction could be more complex. In addition to blocking Na^+ channels, local anesthetics have been shown to bind 1,4-dihydropyridine Ca^{++} channels and depress Ca^{++} entry.[7–10] To our knowledge, it is not been possible to directly measure sensory transduction at the level of nociceptors in skin. However, methods measuring sensory input into the spinal dorsal horn could be used to assess the interaction between local anesthetics and L-type channels in skin nociceptors during radiant heat exposure.

The time-course experiments also revealed that both nifedipine and nicardipine increased the duration of action of bupivacaine. It could be argued that these drugs

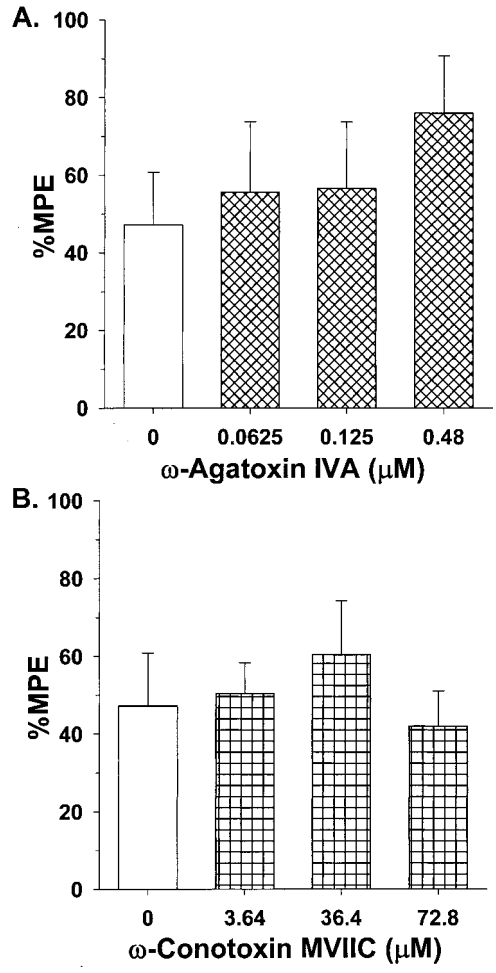

Fig. 9. (*A*) ω-Agatoxin IVA did not affect bupivacaine anesthesia. Baseline tail-flick latencies were obtained before injecting vehicle or ω-agatoxin IVA (0.063–0.48 μM) mixed with 0.12% bupivacaine. Local anesthesia was tested 30 min later. (*B*) ω-Conotoxin MVIIC did not affect bupivacaine anesthesia. Baseline tail-flick latencies were obtained before injecting vehicle or ω-conotoxin MVIIC (3.64–72.8 μM) mixed with 0.12% bupivacaine. The mice were tested for local anesthesia 30 min later. %MPE = percentage of maximum possible effect.

inhibited the local metabolism of bupivacaine. This seems unlikely, because amide-linked local anesthetics undergo hepatic metabolism, and, to our knowledge, bupivacaine does not undergo localized metabolism. In addition, because both nifedipine and nicardipine can cause localized vasodilation,[25,28] the duration of anesthesia should have been reduced if drug redistribution was the major factor affecting duration. For example, subcutaneous infiltration of verapamil or lidocaine in the volar aspect of the arm of human volunteers elicited anesthesia. Yet, when verapamil was infiltrated with lidocaine, the duration of anesthesia was decreased by verapamil increasing localized blood flow.[18] Finally, our data indicate that the enhanced anesthesia was not a result of nifedipine or nicardipine causing vasodilation and increasing the absorption and systemic effects of bupivacaine.

Role of N-, T-, P-, and Q-type Voltage-sensitive Ca^{++} Channels in Bupivacaine Anesthesia

As previously mentioned, the participation of high- and low-voltage–activated Ca^{++} channels in sensory transduction of nociception from skin nociceptors has not been completely addressed. N-type channels appear to be present on small-diameter unmylenated afferent neurons in skin.[24] However, toxins such as ω-conotoxin GVIA that bind the α-1B subunit of N-type Ca^{++} channels[29] have not been tested for skin infiltration studies in animals or humans. Our results indicate that infiltration in the tail of a 100-fold range of ω-conotoxin GVIA concentrations (0.06–6.6 μM) failed to affect bupivacaine anesthesia. In fact, the ED_{50} value of bupivacaine was unaffected by even the 6.6-μM concentration (data not shown). It could be argued that the concentrations were insufficient to exert an effect. However, these concentrations were based on reports in the literature. For example, intophoresis of ω-conotoxin GVIA (1 μM) in lumbar segments 1–4 blocked nociceptive neurons activated by intraarticular kaolin and carrageenan and the response to noxious pressure.[21] In addition, topical application on the spinal dorsal horn from a microdialysis probe (calculated to release 1 μM) prevented the development of secondary hyperalgesia and allodynia to intraplantar injection of capsaicin.[22] Thus, although N-type channels modulate nociceptive input in the spinal cord, their presence on skin nociceptors and participation in sensory transduction were not demonstrated in this assay.

Finally, the role of T-, P-, and Q-type channels in modulating sensory transduction at the level of nociceptors in skin remains to be investigated further. In our hands, the T-type Ca^{++} channel blocker flunarizine failed to modulate bupivacaine anesthesia. In fact, flunarizine was inactive alone, and all three concentrations failed to affect the ED_{50} value for bupivacaine (data not shown). Flunarizine has been reported to block Na^{+} and L-type Ca^{++} channels. Yet flunarizine did not enhance bupivacaine like the 1,4-dihydropyridines or elicit anesthesia like verapamil. Instead, the results indicate that T-type channels, if present on nociceptors, do not modulate bupivacaine anesthesia to noxious radiant heat. Infiltration of the P-type Ca^{++} channel blocker ω-agatoxin IVA (0.063–0.48 μM) in the tail also failed to affect bupivacaine anesthesia. Yet several studies reported that P-type channels in the spinal cord block nociceptive input. Intrathecal ω-agatoxin IVA blocked formalin-induced nociception with an ED_{50} value of 0.1 μM.[30] In addition, ω-agatoxin IVA (0.01–0.1 μM) was found to prevent secondary hyperalgesia and allodynia from intraplantar injection of capsaicin.[22] Our results indicate that P-type channels, if present on nociceptors in skin, do not play a role in modulating bupivacaine anesthesia to radiant heat. Finally, infiltration of the Q-type blocker ω-conotoxin MVIIC (3.6–72.8 μM) also failed to affect bupivacaine anesthesia. These findings are consistent with the observation that spinal application of ω-conotoxin MVIIC (1 or 100 μM) had no effect on responses to noxious pressure in rats injected with intraarticular kaolin-carrageenan.[31]

In summary, Ca^{++} influx through L-type voltage-sensitive channels modulated the anesthetic effects of bupivacaine in a test of radiant heat nociception. The possible role of other voltage-sensitive Ca^{++} channels was ruled out by using selective channel blockers. Thus, L-type Ca^{++} channels on nociceptors in skin appear to play a role in modulating the duration and intensity of bupivacaine anesthesia. Further investigation should seek to identify the participation of high- and low-voltage–activated channels on anesthesia to chemical, pressure, or other forms of nociception.

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