

Amitriptyline for Prolonged Cutaneous Analgesia in the Rat

Mohammed A. Khan, M.D.,* Peter Gerner, M.D.,† Ging Kuo Wang, Ph.D.‡

Background: Amitriptyline has been reported to be a more potent local anesthetic than bupivacaine. In keeping with the objective of identifying drugs for prolonged cutaneous analgesia, the authors compared the cutaneous analgesic effectiveness of amitriptyline and bupivacaine in rats.

Methods: Rats were subcutaneously injected on shaved dorsal skin. The skin wheal raised after injection of 0.6 ml of various concentrations of either amitriptyline or bupivacaine with and without epinephrine (1:200,000) was marked. Inhibition of the cutaneous trunci muscle reflex was evaluated quantitatively by the fraction of times a total of six pinpricks applied to the marked area failed to elicit a nocifensive motor response compared with control responses. No responses out of six pinpricks was defined as 100% maximum possible effect.

Results: Complete recovery from the cutaneous analgesia elicited by 0.05% and 0.5% amitriptyline versus 0.05% and 0.5% bupivacaine occurred in 9.9 ± 0.2 and 19.3 ± 0.4 h versus 2.2 ± 0.1 and 16.1 ± 0.2 h, respectively (mean \pm SE). Addition of epinephrine increased this duration to 14.1 ± 0.1 and 21.4 ± 0.2 h versus 3.2 ± 0.1 and 17.0 ± 0.3 h, respectively. Complete nociceptive blockade after coinjection of 0.25% amitriptyline, 0.25% bupivacaine, and epinephrine lasted 24 ± 0.5 h, and complete recovery from this block took 33 ± 0.5 h. Areas under the percent maximum possible effect versus time curve were $1,770 \pm 24$ and $1,471 \pm 50\%$ h for 0.5% amitriptyline and bupivacaine with epinephrine, respectively, whereas this value was $2,836 \pm 62\%$ h for the coinjected 0.25% amitriptyline, 0.25% bupivacaine, and epinephrine admixture.

Conclusion: Amitriptyline is a longer-acting local anesthetic compared with bupivacaine for cutaneous infiltration. Its analgesic effectiveness is significantly enhanced by epinephrine. Coinjection of amitriptyline and bupivacaine with epinephrine enhances the analgesic duration of both drugs.

INFILTRATION of local anesthetics (LAs) into tissues is an attractive option for surgical anesthesia and management of postoperative pain because it is relatively free of side effects.¹ However, the technique is limited by the short duration of analgesia after infiltration of currently available LAs, which provide analgesia for 3–12 h, whereas wound pain, depending on wound characteristics, lasts for considerably longer.² Bupivacaine, with or without epinephrine, usually is chosen for infiltration because of its longer duration of effective analgesia.^{3,4} Unfortunately, the amount of racemic bupivacaine that can be used, hence the duration of analgesia and the area

covered, is limited by its cardiac and nervous system toxicity.^{2,5-7} Ropivacaine and levobupivacaine offer reduced cardiac toxicity, but the duration of analgesia elicited by these relatively new LAs is comparable to that of bupivacaine.⁸⁻¹¹ Longer-acting LAs that provide a duration of analgesia sufficient for the duration of surgically induced pain would have a significant impact on the practice and economics of postoperative pain management.

The tricyclic antidepressant amitriptyline was recently shown to be a more potent neuronal sodium channel blocker *in vitro* and a more potent and longer-acting LA for rat sciatic nerve blockade *in vivo* compared with bupivacaine.¹² In addition to blocking various voltage-gated Na⁺, K⁺, and Ca⁺ channels,¹³⁻¹⁵ amitriptyline inhibits norepinephrine and serotonin reuptake,¹⁶ blocks α_2 -adrenergic, nicotinic, muscarinic cholinergic, *N*-methyl-D-aspartate, and histaminergic receptors,¹⁷⁻²⁰ and interacts with opioid and adenosine receptors.^{21,22} Numerous studies have shown that amitriptyline effectively decreases pain sensation and thermal hyperalgesia in rats when administered by various routes (oral, intrathecal, peritoneal),²³ and also when combined with opiates and clonidine.^{21,24} Although the exact mechanism by which amitriptyline diminishes pain sensation is not known, overall, its site of action is both central and peripheral.²⁵

To date, amitriptyline has not been reported as a single agent for infiltration anesthesia. We therefore compared the cutaneous analgesic effectiveness of amitriptyline and bupivacaine, with and without epinephrine, after subcutaneous injection in rats as a model for infiltration anesthesia and analgesia. An incidental finding during initial pilot studies showed bupivacaine to have an unexpectedly long duration of analgesia in rats that had previously been injected with amitriptyline. We therefore extended our study to include experiments investigating the effect of subcutaneously coinjecting amitriptyline and bupivacaine, as compared with that of amitriptyline or bupivacaine alone.

Materials and Methods

Chemicals

Both amitriptyline and bupivacaine hydrochloride salts were purchased from Sigma Chemical Co. (St. Louis, MO). Epinephrine was obtained from American Regent Laboratories (Shirley, NY) as a stock solution (1:1000). All drugs were freshly prepared and diluted in a solution of 0.9% NaCl, pH adjusted to 6.5–6.7, within 30 min of administering the injection.

* Research Fellow, † Instructor, ‡ Associate Professor.

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Address reprint requests to Dr. Khan: Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, 75 Francis Street, Boston, Massachusetts 02115. Address electronic mail to: makhan@zeus.bwh.harvard.edu. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

Neurobehavioral Examination

Experiments were performed on conscious, unanesthetized, male Sprague-Dawley rats (weight, 250–300 g). The experimental protocol was approved by the Harvard Medical Area Committee on Animals. All rats were housed on a 12 h light–dark cycle with unlimited access to food and water.

Before the experiments were conducted, the animals were handled daily for up to 14 days to familiarize them with the behavioral investigator, the experimental environment, and the specific experimental procedures. This familiarization minimizes contamination from stress during the experiment and improves experimental performance. Criteria for sufficient handling have been described previously¹¹ and included an absence of behavioral signs of stress (e.g., frequent defecation, immobilization, and lack of exploratory behavior in an open environment) and an extinction of the initially present dorsal contractile response to the nonnoxious stroking of the area to be tested (accommodated by the end of the handling period), followed by a robust, distinctive response to noxious stimulation.

The cutaneous trunci muscle reflex (CTMR), which is characterized by reflex movement of the skin over the back produced by twitches of the lateral thoracic muscles in response to local dorsal cutaneous stimulation, was studied as a reaction to noxious pinprick.²⁶ Because reactions to nonnoxious stroking were intentionally extinguished by repeated handling, it is presumed that responses to stimuli after LA injection were caused by noxious stimulation. The model of infiltration anesthesia used evaluated the inhibition of CTMR produced by subcutaneous injection of the given concentration of drug in 0.6 ml of solution.

After observing the animals normal reaction to six pinpricks applied outside the wheal raised by the drug injections and on the contralateral control side, six pinpricks (at a frequency of 0.5–1 Hz) were applied inside the wheal, and the number to which the rat failed to react was recorded. A Von Frey filament (20.9 g) to which the cut end of an 18-gauge needle was affixed, was used to standardize the stimulus intensity. Six pinpricks per test were sufficient to obtain reproducible results among the different rats within study groups but were few enough to avoid injury (redness, swelling) of the skin during repeated testing of the skin patches studied. The LA effectiveness of the drugs was evaluated quantitatively as the number of times the pinprick failed to elicit a response, with, for example, the complete absence of six responses defined as complete nociceptive block (i.e., 100% of maximum possible effect [MPE]), the absence of three responses of six scored as 50% MPE, and a response identical to the control responses as 0% MPE. The test of six pinpricks was applied every 5–10 min for the first 30 min and then every 15 min to 2 h thereafter until the CTMR fully recovered

from the block. The observer was blinded to the drugs and concentrations used for injections.

Administration of Drugs

The drugs tested were injected (using a 30-gauge needle) subcutaneously in unanesthetized rats under the dorsal surface of the thoracolumbar region, from which hair had been mechanically removed 24 h before the experiments were conducted. The injections caused a circular elevation of the skin, a wheal, approximately 2 cm in diameter. This wheal was marked with ink within a minute after the injection. Each drug (with or without epinephrine) or combination of drugs tested was injected into a naïve area of the rat's shaved back. The back was divided into four quadrants (to clearly demarcate injection and control sites), and each animal was injected twice with the drug being tested, once without epinephrine and once with epinephrine, separated by an interval not less than 5 days. Animals used for the drug combination experiments were given a single injection of a particular drug combination. Because previous studies had shown very dilute solutions of epinephrine and saline to not have analgesic effects when injected alone, we did not include a saline control in our study.²⁶

Rats in the respective groups ($n = 8$ for each group) were injected with a volume of 0.6 ml of the following drugs (weight/volume [%]): (1) 0.05% racemic bupivacaine and 0.05% racemic bupivacaine plus epinephrine (1:200,000); (2) 0.25% racemic bupivacaine and 0.25% racemic bupivacaine plus epinephrine (1:200,000); (3) 0.5% racemic bupivacaine and 0.5% racemic bupivacaine plus epinephrine (1:200,000); (4) 0.05% amitriptyline and 0.05% amitriptyline plus epinephrine (1:200,000); (5) 0.25% amitriptyline and 0.25% amitriptyline plus epinephrine (1:200,000); and (6) 0.5% amitriptyline and 0.5% amitriptyline plus epinephrine (1:200,000).

Each rat in the drug combination groups ($n = 6$ for each group) was injected with a volume of 0.6 ml of the following drugs *via* a single injection (weight/volume [%]): (1) combined 0.05% racemic bupivacaine, 0.05% amitriptyline, and epinephrine (1:200,000); (2) combined 0.125% racemic bupivacaine, 0.125% amitriptyline, and epinephrine (1:200,000); and (3) combined 0.25% racemic bupivacaine, 0.25% amitriptyline, and epinephrine (1:200,000).

Drug combinations were compared in two distinct ways. First, the concentration of the individual drugs in the coinjected combination (durations, area under the curve [AUC], *etc.*) were compared with the sum of the same concentration of the individual drugs injected separately with epinephrine. For example, animals injected with combined 0.05% amitriptyline and bupivacaine with epinephrine, and combined 0.25% amitriptyline and bupivacaine with epinephrine, were compared with the sum of the durations, or the sum of the AUCs of

0.05% amitriptyline with epinephrine and 0.05% bupivacaine with epinephrine injected separately, and with the sum of 0.25% amitriptyline with epinephrine and 0.25% bupivacaine with epinephrine injected separately, respectively. Second, we compared the sum of the individual drug concentrations (or total amount of drug) in the coinjected combinations with the equivalent total amount of individual drugs injected separately with epinephrine. For example, the 0.125% amitriptyline and bupivacaine with epinephrine combination was compared with 0.25% amitriptyline injected separately with epinephrine or with 0.25% bupivacaine injected separately with epinephrine. Similarly, the 0.25% amitriptyline and bupivacaine with epinephrine combination was compared with 0.5% amitriptyline injected separately with epinephrine, or with 0.5% bupivacaine injected separately with epinephrine.

Statistical Analysis

A two-tailed Student *t* test or chi-square test was used as appropriate for establishing significant differences between the values for the different drugs or combinations. Microcal Origin (Microcal Software, Northampton, MA) was used for statistical analysis, calculating areas under the curve, and creating figures. Data were reported as mean \pm SE. Statistical significance was defined as $P < 0.05$.

Results

Amitriptyline and bupivacaine elicited different durations and block densities (*i.e.*, %MPE) of reversible cutaneous analgesia depending on concentration and whether they were administered with or without epinephrine. The comparison of these two drugs or their combination considered their effect on (1) the duration of complete nociceptive blockade, (2) the fraction of animals within a group that had complete nociceptive block at a specified time after injection, (3) the duration until complete recovery of nociception, and (4) the AUC for the %MPE *versus* time plots.

Duration of Complete Nociceptive Blockade

Amitriptyline elicited incomplete nociceptive blockade ($< 100\%$ MPE) in varying fractions of rats at specified times in each group tested. The density of block, averaged over all animals tested and expressed in terms of the maximum graded value, was less than 100% MPE in the amitriptyline groups because not all animals reached a complete nociceptive block for a sustained period (figs. 1A–C), despite there being some time points (especially at higher concentrations) at which block was complete in all animals. In contrast, bupivacaine elicited complete nociceptive blockade (table 1 and figs. 1A–C). Epinephrine significantly ($P < 0.05$, *t* test) increased the

duration of complete nociceptive block at all three concentrations of bupivacaine tested (table 1). When amitriptyline, bupivacaine, and epinephrine were injected in combination (fig. 1D), the duration of complete block was significantly ($P < 0.01$) longer compared with that of bupivacaine with epinephrine alone and reached 24 h in the group that received a combination of 0.25% amitriptyline, 0.25% bupivacaine, and epinephrine (1:200,000) (table 2), whereas the duration was only 11.88 h in the group that received 0.5% bupivacaine plus epinephrine.

Fraction of Animals Completely Blocked between 20 and 40 min after Injection

This measure was scored by recording the number of animals without any response to pinpricks between 20 and 40 min after injection (table 1). The addition of epinephrine significantly increased ($P < 0.05$, chi-square test) the fraction of animals achieving complete block in the amitriptyline groups from 0 to 50% at the 0.05% concentration and from 75 to 100% at the 0.25% concentration, respectively. The block achieved by the higher concentrations of amitriptyline (0.25 and 0.5%) was less reliable than that achieved with the corresponding bupivacaine concentrations; although all individual animals displayed a 100% MPE block at a given point in time between 20 and 40 min after injection (table 1), this effect was not sustained because the very next time point tested may have revealed, depending on the drug concentration, one or two responses to the six pinpricks applied. These “breakthrough” responses in the amitriptyline groups were characteristically in response to the first few pinpricks and were randomly scattered among all the animals tested but markedly decreased by the addition of epinephrine.

Duration until Complete Recovery of Nociception

Amitriptyline produced a significantly longer duration of analgesia at all concentrations compared with bupivacaine (figs. 1A–C). Although the difference in duration until full recovery was highest at the 0.05% concentrations of amitriptyline and bupivacaine (at which the duration was several times longer for amitriptyline) and decreased progressively as the concentrations of both drugs increased, it still remained statistically significant (*t* test, table 1). The ratio between the duration of complete block and full recovery was relatively small for the bupivacaine groups, as can be seen in figures 1A–C, whereas in the amitriptyline groups, this ratio was large because of amitriptyline’s longer duration of less than 100% MPE block. Epinephrine significantly increased ($P < 0.001$, *t* test) the duration until complete recovery in the 0.05% amitriptyline and bupivacaine groups and in the 0.5% amitriptyline group, but not in any of the other groups.

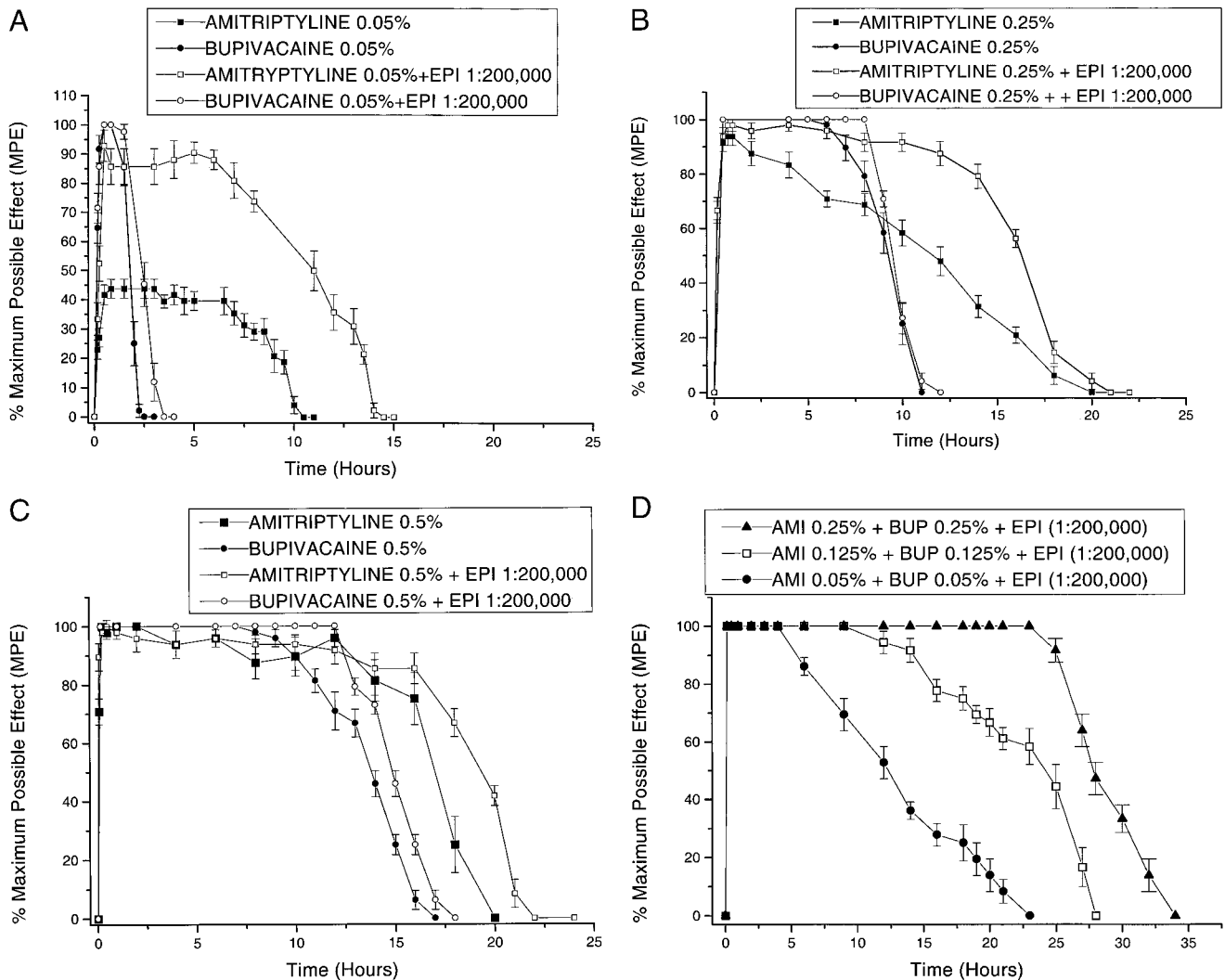


Fig. 1. Comparison of the time course and percent inhibition of the cutaneous trunci muscle reflex, expressed as the graded value of maximum possible effect (MPE) after subcutaneous injections (at time = 0 h) of 0.05% (A), 0.25% (B), and 0.5% (C) concentrations of amitriptyline or bupivacaine with and without epinephrine ($n = 8$ in all groups compared). In (A), 0.05% amitriptyline's sustained partial analgesia (approximately 40–45% MPE) for a prolonged duration is markedly enhanced by epinephrine. (B) Epinephrine retards the rate of block regression of amitriptyline (50% MPE reached at 12.5 h without epinephrine and at 17.5 h with epinephrine) but does not significantly ($P > 0.05$) change the duration until complete recovery of nociception. (C) Differences between the time course of amitriptyline and bupivacaine remain statistically significant but become smaller (compared with the lower concentrations in A and B) as the concentration of the drugs increases. (D) The comparative inhibition of the cutaneous trunci muscle reflex after subcutaneous injections (at time = 0 h) of the combination of amitriptyline, bupivacaine at varying concentrations, and epinephrine ($n = 6$ in all groups compared). The drugs potentiate their analgesic effects as the time courses of the complete nociceptive blockade and the duration until full recovery of nociception are significantly ($P < 0.05$) longer than the time courses of the equivalent total amount (*i. e.*, compared with the total amount of drugs in the combination) of either amitriptyline alone with epinephrine or bupivacaine alone with epinephrine (C). EPI = epinephrine.

When animals were injected with the combination of 0.05% amitriptyline, 0.05% bupivacaine, and epinephrine, the time until full recovery (19.7 h; table 2) significantly outlasted the sum of the time until full recovery of each drug with epinephrine injected alone at the same concentration (17.3 h; $P < 0.05$). Similarly, but statistically not significantly, the combination of 0.25% amitriptyline, 0.25% bupivacaine, and epinephrine had a duration until complete recovery of 33.3 h, which outlasted the sum of the durations until complete recovery of 0.25% amitriptyline with epinephrine and 0.25% bupiv-

acaine with epinephrine injected alone (31.0 h; $P > 0.05$, *t* test). Note that here the comparisons are between the amount of each drug in the coinjected combinations and the sum of the durations of the same amount of each drug injected alone with epinephrine.

The time until full recovery in the groups that received 0.125% and 0.25% amitriptyline and bupivacaine combinations with epinephrine were significantly longer compared with either of the two drugs alone with epinephrine at the 0.25% and 0.5% concentrations, respectively ($P < 0.001$, *t* test; fig. 2). Note that here, the 0.125%

Table 1. Comparison of the Cutaneous Analgesic Duration and Effectiveness of Amitriptyline and Bupivacaine with and without Epinephrine

	Amitriptyline (%)			Bupivacaine (%)		
	0.05	0.25	0.5	0.05	0.25	0.5
Without epinephrine						
Duration of complete block (h)	—	—	—	1.3 ± 0.1	6.5 ± 0.3	9.4 ± 0.6
Time until complete recovery (h)	9.9 ± 0.2*	18.9 ± 0.4*	19.3 ± 0.4*	2.2 ± 0.1	10.6 ± 0.2	16.1 ± 0.2
Fraction of rats fully blocked (%)	0/8 (0%)	6/8 (75%)	8/8 (100%)	7/8 (88%)	8/8 (100%)	8/8 (100%)
AUC (% h)	350 ± 10*	1,034 ± 39*	1,578 ± 18*	162 ± 3	889 ± 27	1,321 ± 55
Plus epinephrine (1:200,000)						
Duration of complete block (h)	—	—	—	1.7 ± 0.1†	7.8 ± 0.1†	11.9 ± 0.1†
Time until complete recovery (h)	14.1 ± 0.1*†	19.8 ± 0.4*	21.4 ± 0.2*†	3.2 ± 0.1†	11.2 ± 0.3	17.0 ± 0.3
Fraction of rats fully blocked (%)	4/8 (50%)‡	8/8 (100%)‡	8/8 (100%)	8/8 (100%)	8/8 (100%)	8/8 (100%)
AUC (% h)	970 ± 12*†	1,512 ± 35*†	1,770 ± 25*†	230 ± 6†	938 ± 28	1,471 ± 50

All values are expressed as mean ± SE, except for fractions. Duration of complete block for amitriptyline is blank because the mean maximum possible effect of all the animals in the respective groups was less than 100%.

* $P < 0.05$ when the area under the curve (AUC) for amitriptyline is significantly more than the same concentration of bupivacaine in the corresponding with-without epinephrine group only. † $P < 0.05$, t test comparison with and without epinephrine. ‡ $P < 0.05$, chi-square test with and without epinephrine.

amitriptyline, bupivacaine, and epinephrine combination (*i.e.*, 0.25% total drugs) is being compared with 0.25% amitriptyline with epinephrine, and with 0.25% bupivacaine with epinephrine; and the combination of 0.25% amitriptyline and bupivacaine with epinephrine (*i.e.*, 0.5% total drug) is being compared with 0.5% amitriptyline with epinephrine, and with 0.5% bupivacaine with epinephrine.

Area under the Percent Maximum Possible Effect versus Time Curve

The analgesic effectiveness of amitriptyline with and without epinephrine is significantly higher than bupivacaine at all the concentrations (with and without epinephrine) when compared with this integrated measure ($P < 0.05$; table 1). Furthermore, epinephrine significantly enhanced ($P < 0.001$) the analgesic effectiveness

of amitriptyline at all the concentrations studied (table 1). The addition of epinephrine increased the AUC of 0.05% amitriptyline by approximately 300% (from 350 to 970% h), but increased the AUC of 0.5% amitriptyline by only 12% (from 1,578 to 1,769% h; table 1). Epinephrine had a significant effect on the analgesic effectiveness of bupivacaine only at the lowest (0.05%) concentration.

The effect of coinjecting amitriptyline, bupivacaine, and epinephrine on integrated analgesic effectiveness is even more pronounced (table 2). Considering first the comparison of each drug injected in combination with the sum of the same amount of drug injected individually with epinephrine, the AUC for the combination of 0.05% amitriptyline, bupivacaine, and epinephrine is not significantly more than the sum of that of the two drugs injected alone with epinephrine at the 0.05% concentration. However, the AUC of the coinjected 0.25% amitrip-

Table 2. Effect of Coinjecting the Amitriptyline, Bupivacaine, and Epinephrine Admixture at Various Concentrations on the Cutaneous Analgesia Elicited

	0.05% Amitriptyline + 0.05% Bupivacaine + 1:200,000 Epinephrine	0.125% Amitriptyline + 0.125% Bupivacaine + 1:200,000 Epinephrine	0.25% Amitriptyline + 0.25% Bupivacaine + 1:200,000 Epinephrine
Duration of complete block (h)	4.3 ± 0.4	12.2 ± 1.1*	24.0 ± 0.5*
Time until complete recovery (h)	19.7 ± 0.7	27.7 ± 0.3†	33.3 ± 0.5†
Fraction of rats fully blocked (%)	6/6 (100%)	6/6 (100%)	6/6 (100%)
Area under the curve (% h)	1,258 ± 42	2,187 ± 52†	2,835 ± 62†

All values are expressed as mean ± SE, except for fractions.

* $P < 0.05$, t test comparison with the value of the corresponding duration of complete block of the equivalent total amount of bupivacaine (*i.e.*, either 0.25 or 0.5%, respectively) injected separately with epinephrine. † $P < 0.05$, t test comparison when the value for the coinjected combination was significantly more than the corresponding value for the equivalent total amount of individual drug (*i.e.*, 0.25 and 0.5%, respectively) of both amitriptyline alone with epinephrine and bupivacaine alone with epinephrine.

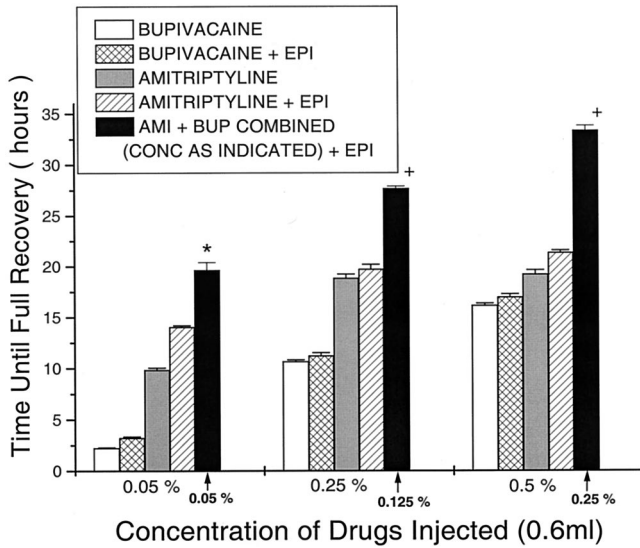


Fig. 2. Comparison of the duration until complete recovery of nociception after injection of either amitriptyline or bupivacaine with and without epinephrine with the corresponding duration after injection of the admixture of amitriptyline, bupivacaine, and epinephrine. For the sake of comparison, the combined concentration of the 0.125 and the 0.25% mixtures of amitriptyline and bupivacaine was kept the same as that of the individual drugs (*i. e.*, 0.25 and 0.5%, respectively). The concentration of amitriptyline and bupivacaine in the mixture is indicated by the arrow. The duration until complete recovery of nociception of the mixture of 0.05% amitriptyline and 0.05% bupivacaine is significantly ($P < 0.05$) greater than the sum of the durations of either drug injected individually at the same concentration with epinephrine. ⁺Duration of the combination was significantly longer ($P < 0.01$) than the durations of the corresponding equivalent total amount of either amitriptyline alone injected with epinephrine or bupivacaine alone injected with epinephrine. EPI = epinephrine.

tyline, 0.25% bupivacaine, and epinephrine combination was significantly higher than the sum of the AUCs of 0.25% amitriptyline injected alone with epinephrine and 0.25% bupivacaine injected alone with epinephrine (tables 1 and 2).

Second, considering the comparison of the sum of the amount of the individual drugs (with epinephrine) in the coinjected combinations (*i.e.*, total amount of the two drugs injected) with the same amount of each individual drug injected separately with epinephrine, the 0.125% amitriptyline, 0.125% bupivacaine, and epinephrine combination had an AUC significantly more than the AUC of either 0.25% amitriptyline injected alone with epinephrine, or 0.25% bupivacaine injected alone with epinephrine ($P < 0.05$; tables 1 and 2). Similarly, the AUC of the 0.25% amitriptyline, 0.25% bupivacaine, and epinephrine combination had an AUC significantly more than either 0.5% amitriptyline injected alone with epinephrine or 0.5% bupivacaine injected alone with epinephrine ($P < 0.05$; tables 1 and 2). The dose-response relations of the integrated analgesic effectiveness of amitriptyline and bupivacaine with and without epinephrine, and in combination, are plotted *versus* concentration in figure 3.

All rats in the amitriptyline, bupivacaine, and combination groups recovered completely and showed no signs of neurobehavioral impairment or of local skin toxicity over a 2-week follow-up period after completion of the experiments.

Discussion

This study describes three principle findings: (1) amitriptyline is an effective and long-acting LA for cutaneous analgesia in our rat model; (2) the cutaneous analgesia

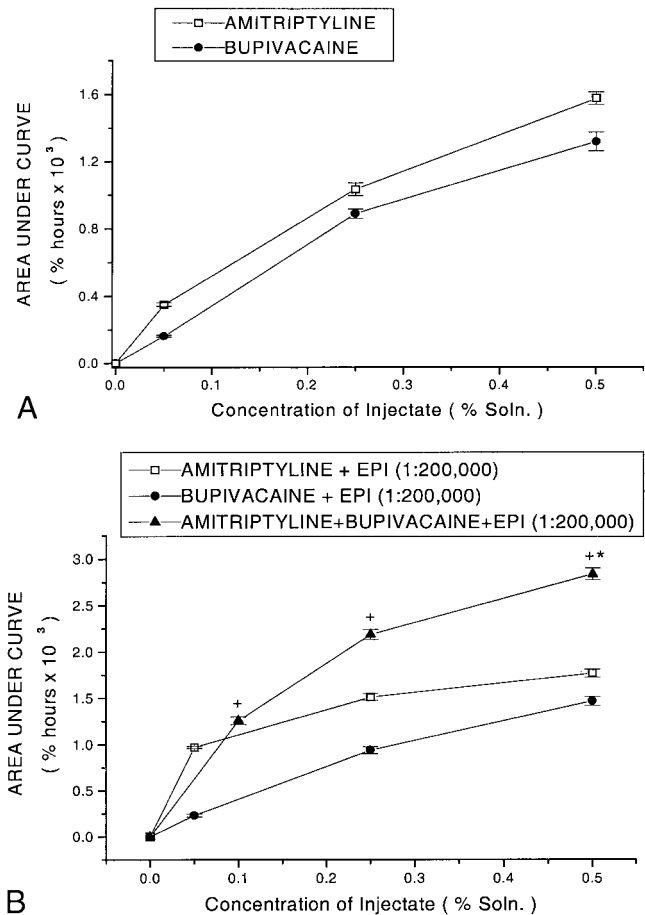


Fig. 3. Dose-response relation of the integrated analgesia (area under the curve for percent maximum possible effect [MPE] *vs.* time plots) elicited after injection of increasing concentrations of amitriptyline and bupivacaine without epinephrine (A) and after injection of amitriptyline or bupivacaine or their combination (admixture) with epinephrine (B). Areas were measured for each rat in the respective groups and then the group mean \pm SE calculated, which was plotted. Injection of the admixture of amitriptyline and bupivacaine combined with epinephrine potentiated the drugs' effects in eliciting cutaneous analgesia. ⁺Area under the curve of the combination is significantly ($P < 0.05$) more than equivalent (same point on x-axis) concentration of either amitriptyline alone with epinephrine or bupivacaine alone with epinephrine. ^{*}Area under the curve of the 0.25% amitriptyline, 0.25% bupivacaine, and epinephrine combination was significantly ($P < 0.05$) more than the sum of the areas under the curve of 0.25% amitriptyline injected alone with epinephrine and 0.25% bupivacaine injected alone with epinephrine. EPI = epinephrine.

elicited by amitriptyline is enhanced by epinephrine; and (3) amitriptyline and bupivacaine potentiate their analgesic effectiveness when subcutaneously coinjected with epinephrine. The significance of these findings is discussed in the following sections.

Amitriptyline Is Applicable for Prolonged Cutaneous Analgesia

We have shown that the duration of cutaneous analgesia (*i.e.*, duration until full recovery of nociception) is longer for amitriptyline than for bupivacaine, either alone or with epinephrine. We also found that amitriptyline is a more effective LA than bupivacaine for tissue infiltration in our rat model when integrated analgesia (AUC) is compared. This is consistent with previous work showing that amitriptyline is a more potent LA than bupivacaine for sciatic nerve blockade in rats.¹² Amitriptyline and desipramine have also been shown to have peripheral antinociceptive actions in the rat paw formalin test, in which these drugs decreased the local pain response after formalin injection.^{22,27} Inhibition of adenosine reuptake was cited as the mechanism underlying amitriptyline's peripheral antinociceptive action in this study, but its LA actions could not be excluded. One of the limitations of our study is that the inhibition of a nocifensive reflex (CTMR) was studied in response to drug injections, and the degree of inhibition of the CTMR was assumed to be indicative of the degree of analgesia.

Enhancement of Cutaneous Analgesia by Epinephrine

The enhancement of cutaneous analgesia elicited by amitriptyline by the addition of epinephrine supports, in part, the vascular uptake hypothesis as a cause of the less dense block of amitriptyline than that of bupivacaine. The antagonism of the vasodilatory effects of amitriptyline by epinephrine²⁸ would slow vascular uptake and increase the concentration locally in the tissues, thus increasing both the duration of the analgesia and the density of the block, as seen in figures 1A-C. The greater impact of epinephrine on the integrated analgesia (AUC) at lower concentrations of amitriptyline probably results from the greater antagonism of the epinephrine-induced vasoconstriction at higher concentrations of amitriptyline. The pharmacokinetics of subcutaneously or intradermally administered bupivacaine and its interactions with vasoconstrictors has been studied extensively and has been discussed elsewhere.^{26,29} Our results are consistent with previous studies showing that epinephrine prolongs the duration of bupivacaine block, depending on the site of administration and the dose of bupivacaine used. Our study showed that epinephrine enhances the cutaneous analgesic effectiveness of amitriptyline to a greater extent than that of bupivacaine, especially at lower concentrations, and that the effect of epinephrine

decreases as the concentration of amitriptyline increases. We chose the epinephrine concentration of 5 $\mu\text{g/ml}$ (1:200,000) because it is the most common clinically used concentration. A study of the effect of varying the concentration of epinephrine on the cutaneous analgesic effectiveness of amitriptyline would be worthwhile.

Potential of Cutaneous Analgesia by Coinjection of Amitriptyline and Bupivacaine

The analgesic effectiveness of amitriptyline and bupivacaine is potentiated by their coinjection in combination with epinephrine. All three parameters—duration of complete nociceptive block, duration until complete recovery of nociception, and the integrated analgesia (AUC)—were significantly greater for the amitriptyline-bupivacaine combinations than for the equivalent amounts of the individual drugs injected separately with epinephrine (tables 1 and 2). In addition, the duration of complete recovery for the 0.05% amitriptyline, bupivacaine, and epinephrine combination was significantly longer than the sum of the durations until complete recovery of 0.05% amitriptyline injected separately with epinephrine, and 0.05% bupivacaine injected separately with epinephrine (fig. 2). Similarly, the AUC of the 0.25% amitriptyline, bupivacaine, and epinephrine combination was significantly more than the sum of the AUCs of 0.25% amitriptyline injected separately with epinephrine and the 0.25% bupivacaine injected separately with epinephrine (tables 1 and 2). This supraadditive analgesic effect of peripherally administered amitriptyline and bupivacaine has not been reported previously. The exact mechanism underlying this effect is not known, but it also was seen in a study comparing the cutaneous analgesic effectiveness of low doses of bupivacaine enantiomers, *i.e.*, the racemic mixture was more effective than the equivalent dose of each enantiomer injected individually.²⁶ Peripheral potentiation of the analgesic effect of infiltrated bupivacaine and lidocaine by the addition of ketamine³⁰ and fentanyl,³¹ respectively, but not of clonidine,³² has also been reported. In these studies, the additives had transient LA properties when injected individually.

Possible explanations for this finding include both pharmacokinetic and pharmacodynamic phenomena. Amitriptyline and bupivacaine could facilitate the transport of one another through diffusion barriers; compete for and displace each other from local protein binding sites, thus increasing the amount of drug available for diffusion into nerves; or retard the dissipation of the coadministered drug into tissues or the bloodstream. Interestingly, the development of tachyphylaxis to the prolonged effects of LAs has been attributed to similar pharmacokinetic factors rather than to pharmacodynamic factors.³³ The prevention of tachyphylaxis to the prolonged LA effects of amitriptyline and bupivacaine

also could account for their potentiating effects after coadministration.

The advantages and disadvantages of combining LAs (e.g., reduced or increased toxicity, rapid onset, prolonged duration, or potentiation) have been discussed elsewhere.³⁴⁻³⁶ Briefly, by using half the concentration of bupivacaine and amitriptyline in the coinjected mixture, and obtaining a significantly longer duration of analgesia than the equivalent total amount of bupivacaine alone with epinephrine (as shown in this study), the concerns about bupivacaine toxicity may be mitigated by using it in combination with amitriptyline and epinephrine. The reduced toxicity, accompanied by the prolonged duration of cutaneous analgesia, could make this combination clinically useful for infiltration anesthesia and postoperative analgesia.

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References

1. Job CA, Fernandez MA, Dorph DJ, Betcher AM: Inguinal hernia repair: Comparison of local, epidural, and general anesthesia. *NY State J Med* 1979; 79:1730-3
2. Cameron AE, Cross FW: Pain and mobility after inguinal herniorrhaphy: Ineffectiveness of subcutaneous bupivacaine. *Br J Surg* 1985; 72:68-9
3. Hashemi K, Middleton MD: Subcutaneous bupivacaine for postoperative analgesia after herniorrhaphy. *Ann R Coll Surg Engl* 1983; 65:38-9
4. Hannibal K, Galatius H, Hansen A, Obel E, Ejlersen E: Preoperative wound infiltration with bupivacaine reduces early and late opioid requirement after hysterectomy. *Anesth Analg* 1996; 83:376-81
5. Davis NL, de Jong RH: Successful resuscitation following massive bupivacaine overdose. *Anesth Analg* 1982; 61:62-4
6. de Jong RH, Bonin JD: Local anesthetics: Injection route alters relative toxicity of bupivacaine. *Anesth Analg* 1980; 59:925-8
7. Nau C, Wang SY, Strichartz GR, Wang GK: Block of human heart hH1 sodium channels by the enantiomers of bupivacaine. *ANESTHESIOLOGY* 2000; 93:1022-33
8. Bay-Nielsen M, Klarskov B, Bech K, Andersen J, Kehlet H: Levobupivacaine vs bupivacaine as infiltration anaesthesia in inguinal herniorrhaphy. *Br J Anaesth* 1999; 82:280-2
9. Erichsen CJ, Vibits H, Dahl JB, Kehlet H: Wound infiltration with ropivacaine and bupivacaine for pain after inguinal herniotomy. *Acta Anaesthesiol Scand* 1995; 39:67-70
10. Johansson B, Glise H, Hallerback B, Dalman P, Kristoffersson A: Preoperative local infiltration with ropivacaine for postoperative pain relief after cholecystectomy. *Anesth Analg* 1994; 78:210-4
11. Vladimirov M, Nau C, Mok WM, Strichartz G: Potency of bupivacaine stereoisomers tested in vitro and in vivo: Biochemical, electrophysiological, and neurobehavioral studies. *ANESTHESIOLOGY* 2000; 93:744-55
12. Gerner P, Mujtaba M, Sinnott CJ, Wang GK: Amitriptyline versus bupivacaine in rat sciatic nerve blockade. *ANESTHESIOLOGY* 2001; 94:661-7
13. Nau C, Seaver M, Wang SY, Wang GK: Block of human heart hH1 sodium channels by amitriptyline. *J Pharmacol Exp Ther* 2000; 292:1015-23
14. Casis O, Sanchez-Chapula JA: Mechanism of block of cardiac transient outward K⁺ current (I_{to}) by antidepressant drugs. *J Cardiovasc Pharmacol* 1998; 32:527-34
15. Joshi PG, Singh A, Ravichandra B: High concentrations of tricyclic antidepressants increase intracellular Ca²⁺ in cultured neural cells. *Neurochem Res* 1999; 24:391-8
16. Sanchez C, Hyttel J: Comparison of the effects of antidepressants and their metabolites on reuptake of biogenic amines and on receptor binding. *Cell Mol Neurobiol* 1999; 19:467-89
17. Gray AM, Pache DM, Sewell RD: Do alpha2-adrenoceptors play an integral role in the antinociceptive mechanism of action of antidepressant compounds? *Eur J Pharmacol* 1999; 378:161-8
18. Park TJ, Shin SY, Suh BC, Suh EK, Lee IS, Kim YS, Kim KT: Differential inhibition of catecholamine secretion by amitriptyline through blockage of nicotinic receptors, sodium channels, and calcium channels in bovine adrenal chromaffin cells. *Synapse* 1998; 29:248-56
19. Eisenach JC, Gebhart GF: Intrathecal amitriptyline acts as an N-methyl-D-aspartate receptor antagonist in the presence of inflammatory hyperalgesia in rats. *ANESTHESIOLOGY* 1995; 83:1046-54
20. Traiffort E, Pollard H, Moreau J, Ruat M, Schwartz JC, Martinez-Mir MI, Palacios JM: Pharmacological characterization and autoradiographic localization of histamine H2 receptors in human brain identified with [125I]iodoaminopentidine. *J Neurochem* 1992; 59:290-9
21. Gray AM, Spencer PS, Sewell RD: The involvement of the opioidergic system in the antinociceptive mechanism of action of antidepressant compounds. *Br J Pharmacol* 1998; 124:669-74
22. Sawynok J, Reid AR, Esser MJ: Peripheral antinociceptive action of amitriptyline in the rat formalin test: Involvement of adenosine. *Pain* 1999; 80:45-55
23. Esser MJ, Sawynok J: Acute amitriptyline in a rat model of neuropathic pain: Differential symptom and route effects. *Pain* 1999; 80:643-53
24. Eisenach JC, Gebhart GF: Intrathecal amitriptyline: Antinociceptive interactions with intravenous morphine and intrathecal clonidine, neostigmine, and carbamylcholine in rats. *ANESTHESIOLOGY* 1995; 83:1036-45
25. Abdi S, Lee DH, Chung JM: The anti-allodynic effects of amitriptyline, gabapentin, and lidocaine in a rat model of neuropathic pain. *Anesth Analg* 1998; 87:1360-6
26. Khodorova AB, Strichartz GR: The addition of dilute epinephrine produces equieffectiveness of bupivacaine enantiomers for cutaneous analgesia in the rat. *Anesth Analg* 2000; 91:410-6
27. Sawynok J, Esser MJ, Reid AR: Peripheral antinociceptive actions of desipramine and fluoxetine in an inflammatory and neuropathic pain test in the rat. *Pain* 1999; 82:149-58
28. Teba L, Schiebel F, Dedhia HV, Lazzell VA: Beneficial effect of norepinephrine in the treatment of circulatory shock caused by tricyclic antidepressant overdose. *Am J Emerg Med* 1988; 6:566-8
29. Aps C, Reynolds F: An intradermal study of the local anaesthetic and vascular effects of the isomers of bupivacaine. *Br J Clin Pharmacol* 1978; 6:63-8
30. Tverskoy M, Oren M, Vaskovich M, Dashkovsky I, Kissin I: Ketamine enhances local anesthetic and analgesic effects of bupivacaine by peripheral mechanism: A study in postoperative patients. *Neurosci Lett* 1996; 215:5-8
31. Tverskoy M, Braslavsky A, Mazor A, Ferman R, Kissin I: The peripheral effect of fentanyl on postoperative pain. *Anesth Analg* 1998; 87:1121-4
32. Elliott S, Eckersall S, Fligelstone L, Jothilingam S: Does the addition of clonidine affect duration of analgesia of bupivacaine wound infiltration in inguinal hernia surgery? *Br J Anaesth* 1997; 79:446-9
33. Choi RH, Birknes JK, Popitz-Bergez FA, Kissin I, Strichartz GR: Pharmacokinetic nature of tachyphylaxis to lidocaine: Peripheral nerve blocks and infiltration anesthesia in rats. *Life Sci* 1997; 61:177-84
34. Cohen SE, Thurlow A: Comparison of a chloroprocaine-bupivacaine mixture with chloroprocaine and bupivacaine used individually for obstetric epidural analgesia. *ANESTHESIOLOGY* 1979; 51:288-92
35. de Jong RH, Bonin JD: Mixtures of local anesthetics are no more toxic than the parent drugs. *ANESTHESIOLOGY* 1981; 54:177-81
36. de Jong RH, Bonin JD: Toxicity of local anesthetic mixtures. *Toxicol Appl Pharmacol* 1980; 54:501-7