

# Amitriptyline versus Bupivacaine in Rat Sciatic Nerve Blockade

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**Background:** Amitriptyline, a tricyclic antidepressant, is frequently used orally for the management of chronic pain. To date there is no report of amitriptyline producing peripheral nerve blockade. The authors therefore investigated the local anesthetic properties of amitriptyline in rats and *in vitro*.

**Methods:** Sciatic nerve blockade was performed with 0.2 ml amitriptyline or bupivacaine at selected concentrations, and the motor, proprioceptive, and nociceptive blockade was evaluated. Cultured rat GH<sub>3</sub> cells were externally perfused with amitriptyline or bupivacaine, and the drug affinity toward inactivated and resting Na<sup>+</sup> channels was assessed under whole-cell voltage clamp conditions. In addition, use-dependent blockade of these drugs at 5 Hz was evaluated.

**Results:** Complete sciatic nerve blockade for nociception was obtained with amitriptyline for 217 ± 19 min (5 mM, n = 8, mean ± SEM) and for 454 ± 38 min (10 mM, n = 7) versus bupivacaine for 90 ± 13 min (15.4 mM, n = 6). The time to full recovery of nociception for amitriptyline was 353 ± 12 min (5 mM) and 656 ± 27 min (10 mM) versus 155 ± 9 min for bupivacaine (15.4 mM). Amitriptyline was approximately 4.7–10.6 times more potent than bupivacaine in binding to the resting channels (50% inhibitory concentration [IC<sub>50</sub>] of 39.8 ± 2.7 vs. 189.6 ± 22.3 μM) at -150 mV, and to the inactivated Na<sup>+</sup> channels (IC<sub>50</sub> of 0.9 ± 0.1 vs. 9.6 ± 0.9 μM) at -60 mV. High-frequency stimulation at 3 μM caused an additional approximately 14% blockade for bupivacaine, but approximately 50% for amitriptyline.

**Conclusion:** Amitriptyline is a more potent blocker of neuronal Na<sup>+</sup> channels than bupivacaine *in vivo* and *in vitro*. These findings suggest that amitriptyline could extend its clinical usefulness for peripheral nerve blockade.

TRICYCLIC antidepressants are commonly used orally in the therapy of chronic pain, such as diabetic neuropathy,<sup>1,2</sup> postherpetic neuralgia,<sup>3</sup> migraine,<sup>4</sup> fibromyalgia and myofascial pain,<sup>5</sup> chronic orofacial pain,<sup>6</sup> central pain, and peripheral neuropathy of different etiology.<sup>7</sup> Among them, amitriptyline has become a mainstay for the treatment of neuropathic pain, which is thought to be caused by an abnormal spontaneous high-frequency ectopic discharge.<sup>8</sup> Amitriptyline was shown to block various voltage-gated ion channels, for example, Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>+</sup> channels.<sup>9–11</sup> Furthermore, it inhibits the reuptake of serotonin and norepinephrine<sup>12</sup>; blocks α<sub>2</sub>-adrenergic, nicotinic, muscarinic cholinergic, N-methyl-

D-aspartate, and histaminergic receptors<sup>13–17</sup>; and interacts with opioid and adenosine receptors.<sup>18,19</sup> Overall, the site of action of amitriptyline is probably both central and peripheral,<sup>20</sup> with a therapeutic plasma concentration of 0.3–0.8 μM.<sup>21</sup>

One of the interesting features of amitriptyline is an additional Na<sup>+</sup> channel blockade (termed as use-dependent or phasic block) at high-frequency stimulation. For example, amitriptyline increased Na<sup>+</sup> channel blockade in isolated rabbit atrial and myocardial myocytes when stimulated at a high frequency<sup>22</sup>; the same held true for voltage-gated Na<sup>+</sup> currents in bovine adrenal chromaffin cells and neonatal dorsal root ganglion cells.<sup>9</sup> This phenomenon of use dependency is also found with clinically used local anesthetics.

Although in numerous reports amitriptyline was shown to effectively decrease the pain sensation, especially for thermal hyperalgesia in rats by various routes of administration (per oral, intrathecal, peritoneal),<sup>23</sup> or when combined with opioids or clonidine,<sup>18,24</sup> the exact mechanism of diminishing the pain sensation is not known. To date, amitriptyline has not been reported as a single agent for peripheral nerve blockade. We therefore compared the effectiveness of amitriptyline and bupivacaine for sciatic nerve blockade in rats. To extend our *in vivo* studies, we also extended former work on the potency and use-dependent blockade of amitriptyline<sup>9</sup> by investigating its voltage-dependent blockade and comparing it to bupivacaine during identical conditions in cultured neuronal cells.

## Material and Methods

### Chemicals

Amitriptyline was purchased from Sigma Chemical Co. (St. Louis, MO); bupivacaine was a gift from AstraZeneca USA, Inc. (Westborough, MA). For the electrophysiological experiments, amitriptyline and bupivacaine were dissolved in dimethyl sulfoxide at 100 mM and were diluted shortly before the experiments. For the sciatic nerve blockade, amitriptyline and bupivacaine hydrochloride were dissolved in 0.9% sodium chloride. On local injection, the low pH of these plain solutions (pH range, 4.9–6.5) is likely to be buffered quickly by the tissue fluid, which has a pH of 7.4.

### Sciatic Nerve Injections

The protocol for animal experimentation was approved by the Harvard Medical Area Standing Committee

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on Animals. Male Sprague-Dawley rats were purchased from Taconic Farm, Inc. (Germantown, NY), and kept in animal housing facilities with controlled room temperature (24°C) and a 12-h (6 AM to 6 PM) light-dark cycle. Rats were handled before behavioral testing to familiarize them with the experiment and to minimize stress-induced analgesia. At the time of injections, animals weighed approximately 250–300 g. The experimenter was blinded to the drug and concentration used.

For sciatic nerve blockade, rats were lightly anesthetized by inhalation of sevoflurane, and the landmarks (greater trochanter and ischial tuberosity) of the left hind limb were localized. A volume of 0.2 ml bupivacaine hydrochloride, 15.4 mM, (corresponding to the frequently used clinical concentration of 0.5%;  $n = 6$ ), 5 mM amitriptyline ( $n = 8$ ), or 10 mM amitriptyline ( $n = 7$ ) was injected in immediate proximity to the sciatic nerve with a 27-gauge hypodermic needle attached to a tuberculin syringe as previously described,<sup>25</sup> and the rat was observed for the development of sciatic nerve block, indicated by complete paralysis of the hind limb. The right hind limb was used as a control.

#### *Neurobehavioral Examination*

Neurobehavioral examination consisted of evaluation of motor function, proprioception, and nocifensive reaction immediately before inhalation of sevoflurane and at 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, and 60 min after the injection, and then at 30-min intervals until 780 min (13.0 h). The following is a brief description of the neurobehavioral examination; details can be found elsewhere.<sup>25</sup>

**Motor function.** Motor function was evaluated by measuring the “extensor postural thrust” of the hind limbs. The rat was held upright with the hind limb extended so that the body weight was supported by the distal metatarsus and toes. The extensor thrust was measured as the gram force applied to a digital platform balance (Ohaus Lopro, Fisher Scientific, Florham Park, NJ), the force that resists contact of the platform by the heel. The preinjection control value (range, 130–165 g) was considered 0% of the maximal possible effect (MPE). The reduction in this force, representing reduced extensor muscle contraction caused by motor blockade, was calculated as a percentage of the control force. A force less than 20 g (also referred to as weight of the “flaccid limb”) was considered 100% MPE.

**Proprioception.** Proprioception evaluation was based on resting posture and postural reactions (“tactile placing” and “hopping”). The functional deficit was graded as 3 (normal) or 0% MPE, 2 (slightly impaired), 1 (severely impaired), and 0 (complete) or 100% MPE.

Hopping response was evoked by lifting the front half of the animal off the ground and then lifting one hind

limb at a time off the ground so that the animal moved laterally. This process normally evokes a prompt hopping with the weight-bearing limb in the direction of movement to avoid falling over. A predominantly motor impairment causes a prompt but weaker than normal response. Conversely, with a predominantly proprioceptive blockade, delayed hopping is followed by greater lateral hops to avoid falling over or, in case of full blockade, no hopping at all.

**Nocifensive reaction.** Nocifensive reaction was evaluated by the withdrawal reflex or vocalization to pinch of a skin fold over the lateral metatarsus (cutaneous pain) and of the distal phalanx of the fifth toe (deep pain). Nocifensive reaction was graded 4 (normal or 0% MPE), 3 (25% MPE), 2 (50% MPE), 1 (75% MPE), and 0 (absent or 100% MPE).

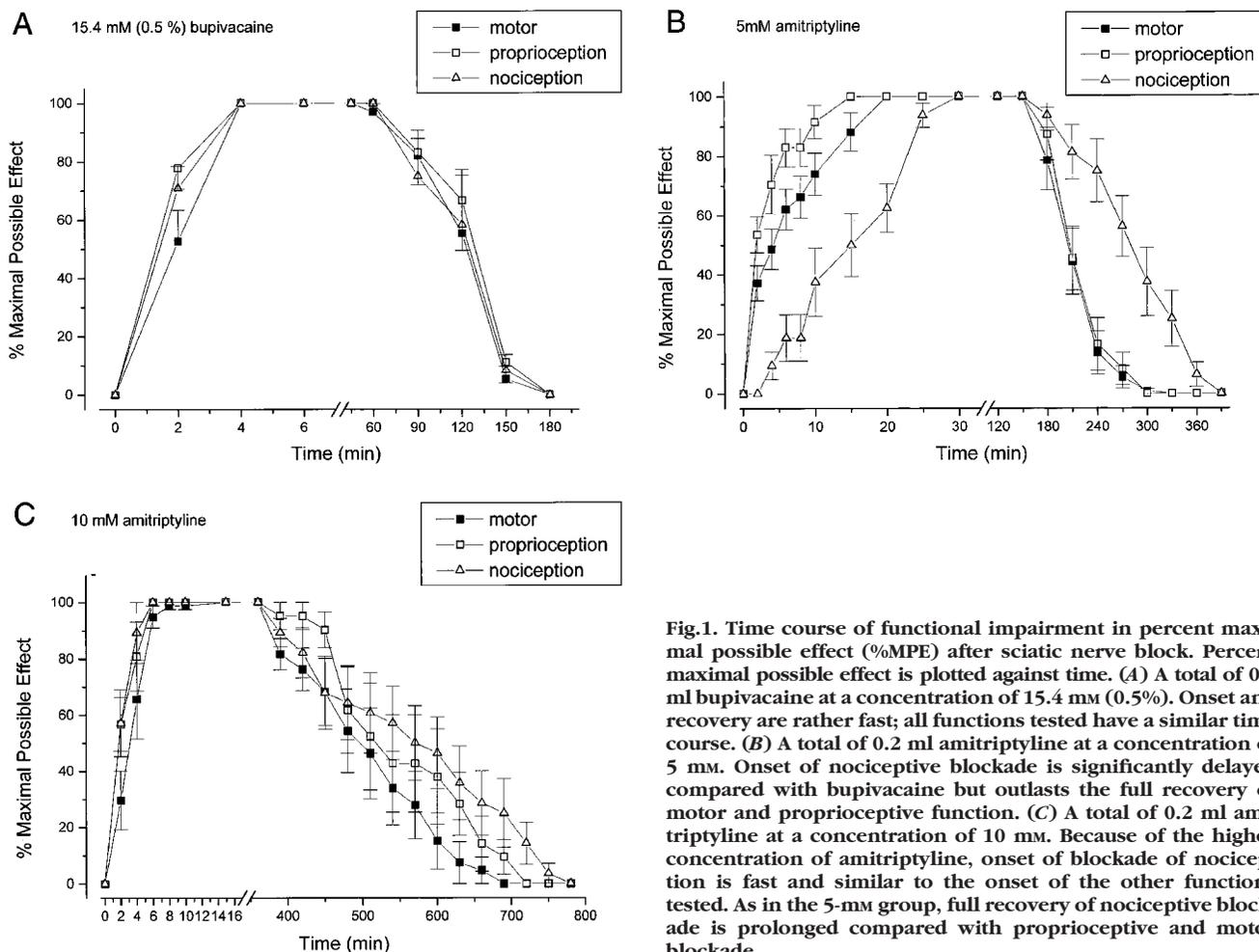
#### *Whole-Cell Voltage Clamp Experiments and Cell Culture*

The whole-cell configuration of the patch clamp technique was used to record macroscopic  $\text{Na}^+$  currents at room temperatures ranging from 21 to 23°C. Pipette electrodes were fabricated with a tip resistance ranging from 0.8 to 1.2 M $\Omega$ . Command voltages were controlled by pCLAMP software (Axons Instruments, Inc., Foster City, CA) and delivered by a List-EPC7 patch clamp amplifier (List-Electronic, Darmstadt/Eberstadt, Germany). Data were filtered at 5 kHz, sampled at 50 kHz, collected, and stored with pCLAMP software. Leak and capacitance currents were subtracted by P/4 protocol, which was not applied in the use-dependent block of  $\text{Na}^+$  currents. Pipette electrodes were filled with an internal solution containing 100 mM NaF, 30 mM NaCl, 10 mM EGTA, and 10 mM HEPES titrated with CsOH to pH 7.2. The external solution consisted of 85 mM choline Cl, 65 mM NaCl, 2 mM  $\text{CaCl}_2$ , and 10 mM HEPES titrated with tetramethylammonium-hydroxide to pH 7.4. Whole-cell recordings can be maintained for more than 1 h in this preparation with little or no run-down of the  $\text{Na}^+$  current.

Rat clonal pituitary GH<sub>3</sub> cells were purchased from the American Type Culture Collection (Rockville, MD). Cells were split twice a week and maintained in Dulbecco modified Eagle medium supplemented with penicillin-streptomycin (1%) and heat-inactivated fetal bovine serum (10%), as previously described.<sup>26</sup>

#### *Statistical Analysis*

An unpaired Student *t* test or a one-way analysis of variance was used to calculate the significance of difference between the 50% inhibitory concentration ( $\text{IC}_{50}$ ) of bupivacaine and amitriptyline or the inhibition of  $\text{Na}^+$  current at the 60th pulse (control, bupivacaine, and



**Fig.1.** Time course of functional impairment in percent maximal possible effect (%MPE) after sciatic nerve block. Percent maximal possible effect is plotted against time. (A) A total of 0.2 ml bupivacaine at a concentration of 15.4 mM (0.5%). Onset and recovery are rather fast; all functions tested have a similar time course. (B) A total of 0.2 ml amitriptyline at a concentration of 5 mM. Onset of nociceptive blockade is significantly delayed compared with bupivacaine but outlasts the full recovery of motor and proprioceptive function. (C) A total of 0.2 ml amitriptyline at a concentration of 10 mM. Because of the higher concentration of amitriptyline, onset of blockade of nociception is fast and similar to the onset of the other functions tested. As in the 5-mM group, full recovery of nociceptive blockade is prolonged compared with proprioceptive and motor blockade.

amitriptyline). An unpaired Student *t* test was also used to detect significant differences among the proprioceptive, motor, and nociceptive functions of the animals after bupivacaine or amitriptyline injection (Origin, Microcal Software, Inc., Northampton, MA). Data are presented as mean ± SE. Statistical significance was defined as *P* < 0.05.

**Results**

*Rat Sciatic Nerve Blockade*

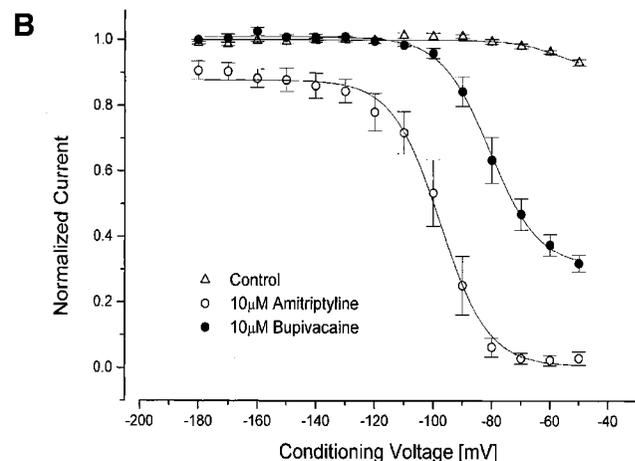
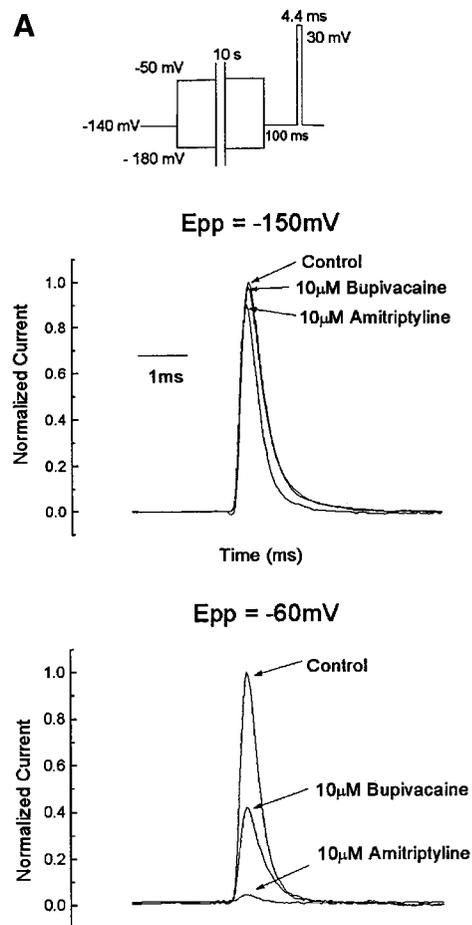
All rats developed a complete sciatic nerve blockade after the amitriptyline injection. The detailed time course of onset and recovery of blockade is shown in figures 1A–1C. All animals recovered promptly from sevoflurane inhalation anesthesia (~1–1.5 min), allowing also to examine the onset of the block.

**Duration of Complete Blockade.** In the 15.4-mM bupivacaine group, differential blockade was not observed (90 ± 13 min for blockade of proprioception and nociception, 88 ± 15 min for motor blockade). In the 5-mM, but not in the 10-mM amitriptyline group, nociceptive blockade was statistically significantly longer than

motor blockade (218 ± 19 and 455 ± 38 vs. 169 ± 8 and 403 ± 28 min, respectively).

**Duration to Full Recovery.** In contrast to the 15.4-mM bupivacaine group, where all functions tested were recovering roughly at the same time, for the animals in the amitriptyline groups, recovery of nociceptive blockade was delayed compared with motor and proprioceptive blockade. The time to full recovery of functions for amitriptyline at 5 and 10 mM was 353 ± 12 and 656 ± 27 for nociception, 270 ± 14 and 583 ± 33 for motor function, 255 ± 11 and 579 ± 40 min for proprioception and for bupivacaine 155 ± 9, 160 ± 6, and 155 ± 9 min, respectively. Therefore, within the 5-mM amitriptyline group, nociceptive function was blocked significantly longer than motor and proprioceptive function.

It is noteworthy that the time of complete blockade of functions and the time to full recovery of functions are calculated differently from figure 1. For example, in the 5-mM amitriptyline group at 210 min, five rats showed no nocifensive response, one showed 25%, and two showed 50% nocifensive reaction, which amounts to 81.3 ± 9.1% MPE (fig. 1B; n = 8). On the other hand, the time of complete nociceptive blockade was 150, 150, 180,



**Fig. 2.** Voltage-dependent blockade with amitriptyline and bupivacaine at a concentration of  $10 \mu\text{M}$  or with no drug (control). **(A)** Pulse protocol and representative tracings. The holding potential was  $-140 \text{ mV}$ , followed by a 10-s prepulse or conditioning pulse (intended to be long enough to allow binding to reach steady state at the prepulse potential). The prepulse potential was stepped up from  $-180 \text{ mV}$  to  $-50 \text{ mV}$  in 10-mV steps. After this prepulse or conditioning pulse, the membrane potential was stepped back to the holding potential ( $-140 \text{ mV}$ ) for 100 ms, which allowed drug-free channels to recover from fast inactivation. The second pulse (test pulse) to  $30 \text{ mV}$  elicits the outward transient flow of  $\text{Na}^+$  current in drug-free channels; the relative magnitude thereof is used to determine the percentage of channels that were not bound with drug during the conditioning pulse. Pulses were delivered at 40-s intervals. Representative tracings for a drug concentration of  $10 \mu\text{M}$  are shown for the resting state ( $E_{\text{pp}} = -150 \text{ mV}$ ) and for the inactivated state (prepulse potential =  $-60 \text{ mV}$ ). **(B)** Normalized  $\text{Na}^+$  current in the absence (control) or presence of amitriptyline and bupivacaine at a concentration of  $10 \mu\text{M}$  at various conditioning voltages. Data were fitted with a Boltzmann function ( $1/[1 + \exp((V_{0.5} - V)/k_E)]$ ).

The average  $V_{0.5}$  values (50% availabilities) and  $k_E$  values (a slope factor) for the fitted Boltzmann functions were  $-97.6 \pm 0.8$  and  $8.1 \pm 0.7 \text{ mV}$  for amitriptyline,  $-80.1 \pm 0.4$  and  $8.2 \pm 0.4 \text{ mV}$  for bupivacaine, and  $-57.3 \pm 11.7$  and  $7.3 \pm 5.1 \text{ mV}$  for the control.

210, 240, 240, 270, and 300 min, which amounts to  $218 \pm 19 \text{ min}$ .

All rats in the amitriptyline and bupivacaine groups recovered completely and showed no signs of neurobehavioral impairment. Therefore, histopathologic studies were not included in this work.

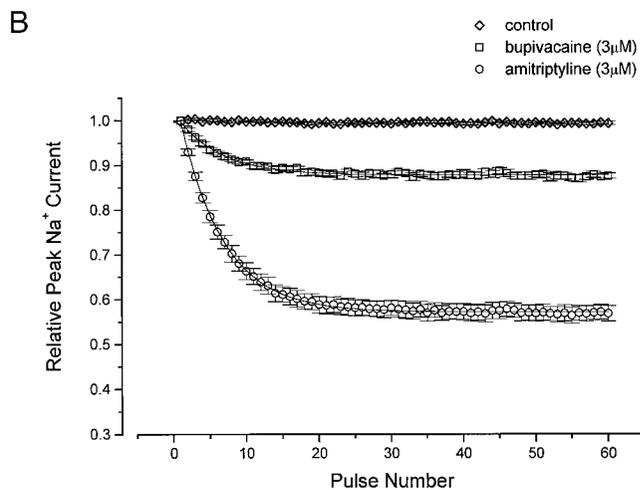
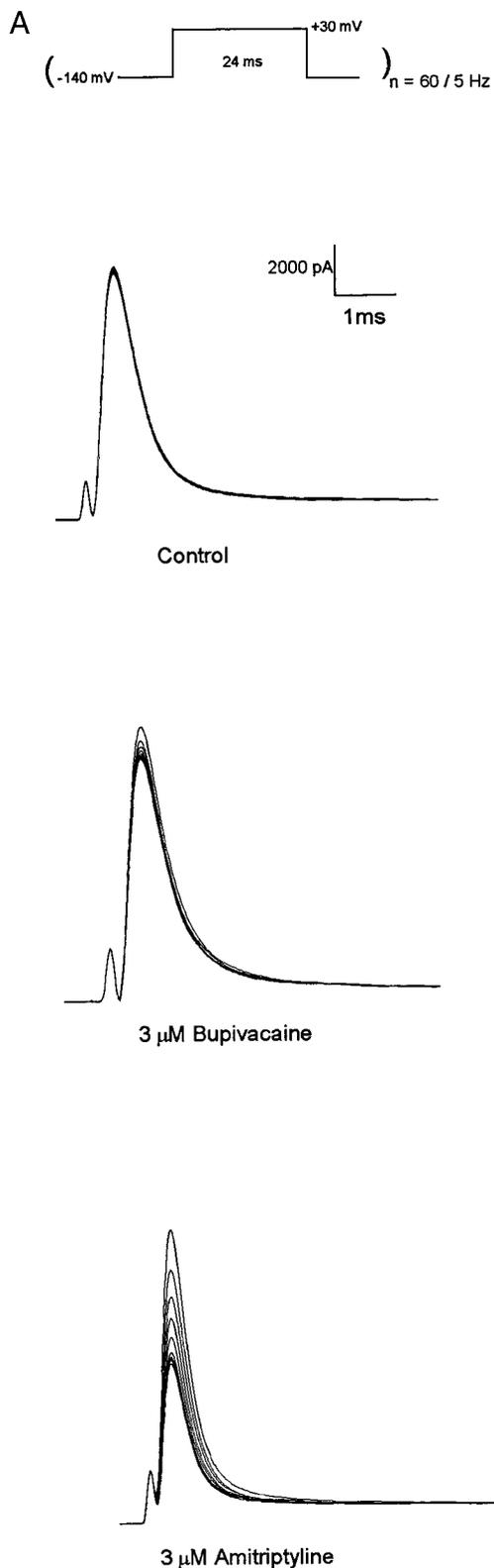
#### Single-Cell Studies

To determine the voltage-dependent blockade by amitriptyline or bupivacaine, a prepulse or conditioning pulse at various voltages long enough to permit the drug-channel binding interaction to reach its steady state level<sup>27</sup> was applied (pulse protocol and representative tracings are shown in fig. 2A). The blocking characteristics at different voltages for rat clonal pituitary GH<sub>3</sub> cells with drug application (amitriptyline or bupivacaine) or without drug (control) are shown in figure 2B. Both drugs reach asymptote at a conditioning voltage of  $-150$  and  $-60 \text{ mV}$ . To determine the potency of amitriptyline and bupivacaine for the resting and inactivated states, dose-response curves were subsequently constructed at conditioning potentials of  $-150$  and  $-60 \text{ mV}$ , respec-

tively. Finally, additional blockade provoked by high-frequency stimulation (use-dependent blockade) was investigated (pulse protocol and representative tracings are shown in fig. 3A). Five cells were used for each drug concentration or for control.

**Affinity for Resting and Inactivated Channels.** Dose-response curves revealed that in the inactivated state ( $-60 \text{ mV}$ ) amitriptyline was approximately 10.6 times more potent, and in the resting state ( $-150 \text{ mV}$ ) was approximately 4.7 times more potent than bupivacaine. The  $\text{IC}_{50}$  values of amitriptyline and bupivacaine at  $-150 \text{ mV}$  were  $39.8 \pm 2.7$  and  $189.6 \pm 22.3$  and at  $-60 \text{ mV}$  were  $0.9 \pm 0.1$  and  $9.6 \pm 0.9 \mu\text{M}$ , respectively ( $P < 0.05$ ). The Hill coefficient was calculated for amitriptyline and bupivacaine in the resting state as  $1.42 \pm 0.12$  and  $0.86 \pm 0.07$ , and in the inactivated state as  $1.35 \pm 0.15$  and  $1.11 \pm 0.11$ , respectively.

**Use-dependent Blockade.** High-frequency stimulation at 5 Hz produced no measurable blockade of  $\text{Na}^+$  currents in the control (fig. 3B). External perfusion of the cells with a  $3\text{-}\mu\text{M}$  concentration of bupivacaine caused an additional approximately 14% blockade during



**Fig. 3.** Use-dependent block of amitriptyline and bupivacaine. (A) Pulse protocol and representative tracings. Holding potential was  $-140$  mV, followed by a test pulse to  $+30$  mV. Duration of the test pulse was 24 ms. This cycle was obtained for a total of 60 pulses at a frequency of 5 Hz. Tracings are shown at pulse number 1, 3, 5, 7, 10, 15, 20, 30...60 for control as well as amitriptyline and bupivacaine. (B) After steady state was obtained at a concentration of  $3 \mu\text{M}$  for amitriptyline and bupivacaine, the consecutive high-frequency stimulation revealed an additional block of  $50.3 \pm 0.3\%$  for amitriptyline versus  $14 \pm 0.3\%$  for bupivacaine. Even at high use-dependent stimulation, no block was achieved when no drug was applied. The data were well fitted by a single exponential function with a time constant of  $5.9 \pm 0.05$  pulse for amitriptyline and  $6.0 \pm 0.2$  pulse for bupivacaine.

**Discussion**

We have shown that amitriptyline is a much more potent  $\text{Na}^+$  channel blocker than bupivacaine *in vivo* as well as *in vitro*. Sciatic nerve blockade was approximately 2.3 times longer with amitriptyline at 5 mm and 4.2 times longer with amitriptyline at 10 mm for full recovery of nociception, although the concentration of bupivacaine at 15.4 mm was approximately 3.1 and 1.5 times higher, respectively. The  $\text{IC}_{50}$  of amitriptyline for tonic block in rat  $\text{GH}_3$  cells is in agreement with former work in neuroendocrine cells by Pancrazio *et al.*<sup>9</sup> In addition, we found that the potency of amitriptyline is dependent on the  $\text{Na}^+$  channel state (approximately 4.7–10.6 times higher than that of bupivacaine). Amitriptyline also shows approximately 3.5 times more use-dependent block at  $3 \mu\text{M}$ . These data confirm that amitriptyline also has local anesthetic properties and clearly demonstrate that amitriptyline is an even more potent local anesthetic than bupivacaine for sciatic nerve block in rats. Bupivacaine was chosen for comparison as it is the local anesthetic of choice for most anesthesiologists when a long-lasting block with predominance of sensory over motor blockade is desired.

*Mode of Action of Amitriptyline*

In general, it is thought that the therapeutic site of action of amitriptyline is predominantly central and

repetitive stimulation, whereas a  $3\text{-}\mu\text{M}$  concentration of amitriptyline caused approximately 50% blockade (fig. 3B). The differences among these three groups are statistically significant ( $P < 0.05$ ).

mainly consists of norepinephrine reuptake inhibition<sup>12</sup> and *N*-methyl-D-aspartate<sup>16</sup> and  $\alpha_2$ -adrenergic antagonism.<sup>13</sup> Although we provided evidence of the Na<sup>+</sup> current-inhibiting effects of amitriptyline, it is less clear whether this mechanism is clinically relevant in terms of treatment of chronic pain. Several studies compared lidocaine with amitriptyline. In studies with human volunteers and intradermal capsaicin injection (which is thought to lead to the development of hypersensitivity of dorsal horn neurons to afferent input by stimulation of *N*-methyl-D-aspartate and other excitatory receptors), intravenous lidocaine was found to decrease all secondary hyperalgesia responses,<sup>28</sup> but pain report was unaffected after 25 mg amitriptyline administered intramuscularly.<sup>29</sup> Similarly, in patients suffering from neuropathic pain (the underlying mechanism is thought to be an increase in the density of Na<sup>+</sup> channels in the neuroma and dorsal root ganglion of the injured axon), infusion of lidocaine leads to a plasma concentration-dependent acute reduction of spontaneous pain,<sup>30,31</sup> whereas the clinical impression is that amitriptyline needs several weeks to become effective. These obvious discrepancies could be explained on the basis of plasticity of Na<sup>+</sup> channel expression in a regionally and temporally specific manner, with different Na<sup>+</sup> channel subtypes having different distributions and downregulation of certain Na<sup>+</sup> channel genes and upregulation of previously silent genes.<sup>32</sup> Alternatively, because amitriptyline (as well as lidocaine) exerts many effects on various ion channels and receptors, it is feasible that, dependent on the specific disease or experimental set-up, amitriptyline and lidocaine cause pain relief by a mechanism other than Na<sup>+</sup> channel blockade.

#### *Amitriptyline In Vitro*

**Voltage-dependent Blockade.** Our results support a highly significant state-dependent blockade of Na<sup>+</sup> channels by both amitriptyline and bupivacaine. The preferential binding of amitriptyline to Na<sup>+</sup> channels in the inactivated state is in agreement with earlier work with a different Na<sup>+</sup> channel isoform.<sup>9,33</sup> Based on these results, we therefore constructed the dose-response curves for amitriptyline and bupivacaine at  $-150$  mV for the resting and at  $-60$  mV for the inactivated state.

#### *Use-dependent Block.*

The results of our use-dependency studies are in agreement with earlier work in bovine adrenal chromaffine cells,<sup>9</sup> cardiac myocytes,<sup>22</sup> Purkinje fibers,<sup>34</sup> and neuroblastoma cells.<sup>35</sup> The high level of use-dependent blockade in this current work is also found in the human heart hH1 Na<sup>+</sup> channel isoform,<sup>33</sup> which at least partly explains the cardiotoxicity of this drug. These findings together with our findings in regard to the voltage-dependent effects of amitriptyline are supported by an *in vivo* study with amitriptyline, which produced a sub-

stantial state-dependent conduction slowing within the His-Purkinje system.<sup>36</sup> Although the phenomenon of use-dependent blockade clearly adds to the cardiotoxicity of this drug, it is also likely beneficial for the treatment of pain states with a high rate of action potential discharge, e.g., acute postoperative and neuropathic pain.

#### *Amitriptyline In Vivo*

As previously mentioned, amitriptyline is widely used for the management of chronic pain. To date, a literature search has revealed no reports of the use of amitriptyline as a single agent for peripheral nerve blockade, but it has been extensively described as a single agent or in combination with other agents for intrathecal application in different species.<sup>37,38</sup>

We found amitriptyline to be much more potent than bupivacaine, especially for nociceptive block (which for amitriptyline at 10 mM was 4.2 times longer than for bupivacaine at 15.4 mM). In a previous study, amitriptyline administered intrathecally in rats had no effect on nociception,<sup>24</sup> which is contradictory to the effectiveness of amitriptyline in sciatic nerve blockade. However, considering that in this study an overall much lower dosage was used—60  $\mu$ g of amitriptyline in 3  $\mu$ l (which is approximately a concentration of 20 mM)—this could be explained by reasons of dosage difference alone, as in our study 200  $\mu$ l of a 5-mM concentration was used. It also seems that amitriptyline is not entirely without effect even at such a relatively low dosage as “spinal administration of amitriptyline (60  $\mu$ g) produced an antihyperalgesic effect” in a rat model of neuropathic pain.<sup>23</sup> In addition, amitriptyline might be less effective on the spinal cord *per se* or different pharmacokinetics, for example, a relative high solubility in cerebrospinal fluid because of hydrophilicity, could further decrease the effectivity of amitriptyline when administered intrathecally. A similar argument could be made for the only brief period of antinociception after 5 mg cervical amitriptyline administered intrathecally in sheep.<sup>38</sup> Of note, 2 days later sheep received 10 mg amitriptyline according to the same protocol and showed intense sedation with minimal or no response to noise or antinociception testing, and one of the four sheep had a generalized seizure 5 min after injection and died. This of course raises the concern that further work on amitriptyline might not be warranted, but the death of this sheep could rather be the direct effect of the drug injected very close to the medulla oblongata with consecutive cardiorespiratory arrest, executed through the local anesthetic properties of amitriptyline in a way similar as one would expect with a comparable dose of lidocaine.

In summary, amitriptyline is a potent Na<sup>+</sup> channel blocker *in vivo* and *in vitro*. In rat sciatic nerve block, the time to full recovery of nociceptive response is longer than blockade of motor function. Because it has a similar affinity for the human heart as for neuronal Na<sup>+</sup> channels, cardiac

toxicity is a potentially fatal effect of parenteral administration. This risk could be decreased by local injection, topical application, or modification of the parent drug.

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