ELSEVIER

European Journal of Pharmacology 439 (2002) 77–81

ejp

www.elsevier.com/locate/ejphar

Comparison of nerve conduction blocks by an opioid and a local anesthetic

Tufan Mert [a], Yasemin Gunes [b], Mustafa Guven [a], Ismail Gunay [a,*], Dilek Ozcengiz [b]

[a] *Department of Biophysics, School of Medicine, University of Cukurova, 01330 Balcali, Adana, Turkey*
[b] *Department of Anesthesiology, School of Medicine, University of Cukurova, 01330 Balcali, Adana, Turkey*

Received 4 October 2001; received in revised form 6 February 2002; accepted 8 February 2002

Abstract

The experiments were done on frog sciatic nerves, using a sucrose-gap recording technique. The aim of our study was to investigate and to compare the tonic and phasic conduction blocking potency of tramadol and lidocaine on whole nerve and their interactions with Ca^{2+}. The concentration of a tramadol solution producing the same amount of tonic and phasic conduction blocks was three and six times higher than that needed for lidocaine, respectively. Increasing the Ca^{2+} concentration in the test solution enhanced the conduction blocking potency of tramadol, but decreased that of lidocaine. It is concluded that tramadol blocks nerve conduction like a local anesthetic but with a weaker effect than that of lidocaine. Interactions of Ca^{2+} and these drugs suggested that these drugs might have either different binding sites or different action mechanisms.

Keywords: Lidocaine; Tramadol; Sucrose-gap; Compound action potential; Sciatic nerve; (Frog)

1. Introduction

Local anesthetic agents are known to block impulse conduction by inhibiting voltage-dependent Na^+ channels in a concentration-dependent manner. Additionally, local anesthetics are capable of changing nerve conduction without affecting resting membrane potential. They bind more effectively to the open and inactivated states than to the resting states of Na^+ channels. Therefore, in addition to the tonic block observed at low conduction frequencies, these drugs produce an additional block at high conduction frequencies, called phasic block (Bokesh and Strichartz, 1986; Drachman and Strichartz, 1991; Mert and Gunay, 1999).

Inhibition by local anesthetics to produce Na^+ channel block was also demonstrated for some substances that have local anesthetic effects, such as ketamine and meperidine (Hunter and Frank, 1979; Brau et al., 1997). It is postulated that meperidine has a local anesthetic-like activity because of its Na^+ channel blocking potency, but its affinity to peripheral nerve Na^+ channels is lower than that of lidocaine. However, different K^+ channels of peripheral nerves can also be inhibited by meperidine (Brau et al., 2000). Tramadol, a synthetic opioid in the aminocyclohexanol group, is a centrally acting analgesic. It is postulated that it has a local anesthetic effect similar to that of lidocaine following intradermal injection (Pang et al., 1999; Roux and Coetzee, 2000). No effect of tramadol on in vitro preparations has, however, been shown so far.

It is recognized that Ca^{2+}, a divalent cation, plays a role in nerve excitability. It may induce changes in nerve conduction without any considerable alteration of resting membrane potential, similarly to the influence of local anesthetics (Saito et al., 1984). Raising the external Ca^{2+} concentration increases the threshold for electrical excitation and closes voltage-dependent channels. Thus, raising external Ca^{2+} concentration causes a decrease in inward Na^+ current in nerves (Hille, 1992).

The aim of this research was to investigate the interaction of tramadol, Ca^{2+} and conduction frequency on compound action potentials of whole frog sciatic nerve and compare these effects with those of lidocaine.

2. Materials and methods

2.1. Preparation

In this study, sciatic nerve bundles, 4–5 cm in length, were taken from frogs (*Rana cameranoi*), weighing 70–80

* Corresponding author. Tel.: +90-322-3387105; fax: +90-322-3386847.
E-mail address: igunay@mail.cu.edu.tr (I. Gunay).

0014-2999/02/$ - see front matter
PII: S0014-2999(02)01368-7

g. The frogs were rapidly decapitated and then sciatic nerves were dissected from the lumbar plexus to the knee. The nerves were kept in normal Ringer's solution at 4–6 °C for a day before the start of experiment.

2.2. Solutions

Normal frog Ringer's Solution (mM): NaCl 114; KCl 2; $CaCl_2$ 1.9; $NaHCO_3$ 10; glucose 5.5. Isotonic KCl solution (mM): NaCl 2; KCl 114; $CaCl_2$ 1.9; $NaHCO_3$ 10; glucose 5.5. Isotonic sucrose solution: 245 mM sucrose. Test Solutions: 1.6, 3.3, 6.6, 9.9 mM tramadol and 1, 2.1, 4.2 mM lidocaine. Tramadol (6.6 mM) and lidocaine (1 mM) solutions were prepared in Ringer's solution with 3.8, 1.9 mM Ca^{2+} and without Ca^{2+}. Deionised and bi-distilled water were used for the solutions. In preparations, all solutions were bubbled with a 95% O_2 and 5% CO_2 gas mixture. The pH of the solutions was adjusted to 7.4 with NaOH and HCl, if needed.

2.3. Stimulation and recording instruments

A Grass S48 stimulator and stimulus isolation unit (SIU5), Grass P16 microelectrode AC/DC amplifier, Hitachi VC-6523 digital storage oscilloscope, Cole Parmer pen recorder with two channels, and a Master flex perfusion pump with eight channels and A/D card + computer were used in the experiments.

Before the experiments, the sciatic nerves were desheathed and then placed in a sucrose-gap apparatus for stimulation and recording (Fig. 1). For the experiments, a nerve was stimulated with voltages 1.5 to 2 times supramaximal for 0.05-ms duration. When the control values were recorded, the nerve was stimulated as tonic with a single stimulus or as phasic using repetitive stimuli with a 10-Hz train, lasting 1000 ms and a 40-Hz train, lasting 500 ms, respectively (Fig. 2A).

After the control values were recorded, test solutions were added into the test pool, the nerve was stimulated once per 5 min (tonic stimulation) for 35 min and the responses were recorded. At the end of this period, the compound action potential (CAP) recorded was taken as a non-frequency-dependent response (tonic response). Frequency-dependent responses (phasic response) were recorded, according to the protocol described above (Fig. 2B). All records were transferred to a computer to be evaluated later. Test solutions were applied when the nerves had a control compound action potential amplitude of over 35 mV and six nerve preparations were used for each drug.

2.4. Statistical analysis

Normalized compound action potential values for changes with all test solutions were reported as percentages of control amplitude (mean ± S.E.M). Differences due to test solutions were tested for significance with an unpaired Student's *t*-test. Significance was set at $P < 0.001$.

3. Results

In the experiments, the blocks produced by test solutions on compound action potential amplitude was calculated using the formula shown in Fig. 2C. As shown in Fig. 2B, the maximum blocking effect was observed after the seventh or eighth stimulating pulse.

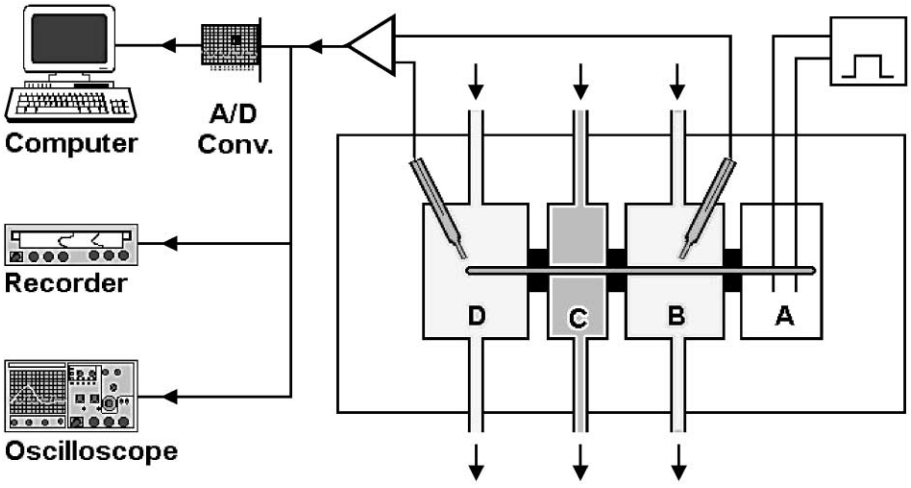

Fig. 1. Diagram of sucrose-gap recording technique: sucrose-gap apparatus having four pools made by using Plexiglas (Stampfli, 1954; Strong et al., 1973). Pool A, containing a pair of platinum stimulating electrodes, is filled with mineral oil to protect nerves from drying; Pool B contains Ringer's or test solution; Pool C contains nonionic isotonic sucrose and Pool D contains isotonic KCl solution. Pools are isolated from each other with vaseline–silicon oil mixture. The potential difference between Pools B and D was recorded by using agar bridge Ag–AgCl electrodes (Marsh, 1989). All solutions were perfused at the rate of 2–3 ml/min. All experiments were carried out at room temperature (21–23 °C).

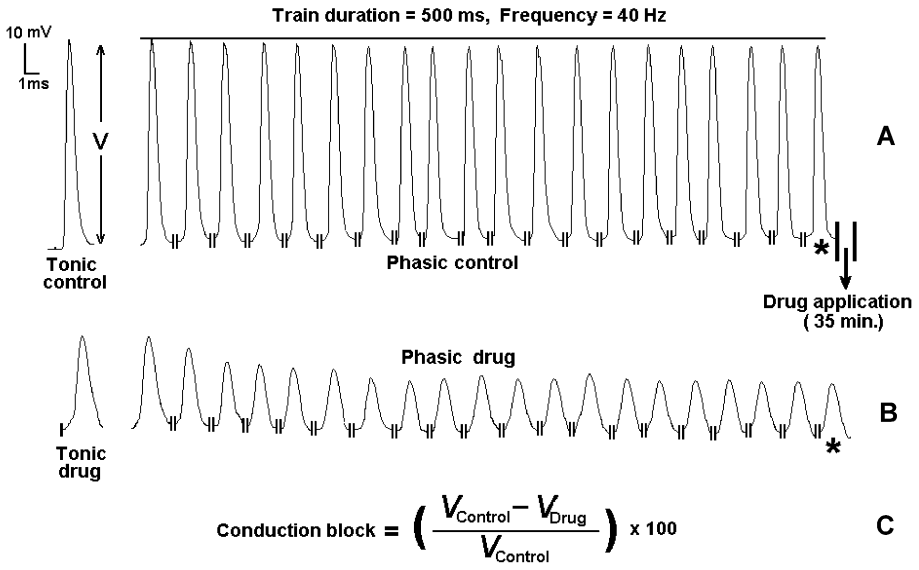

Fig. 2. A typical record used to calculate tonic and phasic conduction blocks. (A) Tonic and phasic control records. The tonic control (drug-free) is nerve response (compound action potential) to a single stimulus. The phasic control is nerve response to a 40-Hz train lasting 500 ms. (B) Tonic and phasic responses following the 35 min after the drug application. (C) The formula used to calculate nerve conduction block. Tonic block is defined as the percentage of relative decrease in the amplitude (V) of compound action potential. Phasic block is the percentage of relative decrease in amplitude (V) of the last pulses of trains (marked with asterisk, *).

3.1. Comparison of blocks produced by tramadol and lidocaine

3.1.1. Tonic conduction blocks

When tramadol and lidocaine concentrations were increased in the test solution, their tonic conduction blocks also increased (Fig. 3). Tonic block for tramadol was 14.3% at 1.6 mM concentration and 67.7% at 9.9 mM concentration; for lidocaine, it was 37.7% at 1 mM concentration and 98% at 4.2 mM concentration.

ED_{50} values were 2.2 mM for lidocaine and 6.6 mM for tramadol (Fig. 3). Three-fold higher tramadol concentrations had to be used to produce the same level of tonic conduction blocks as with lidocaine.

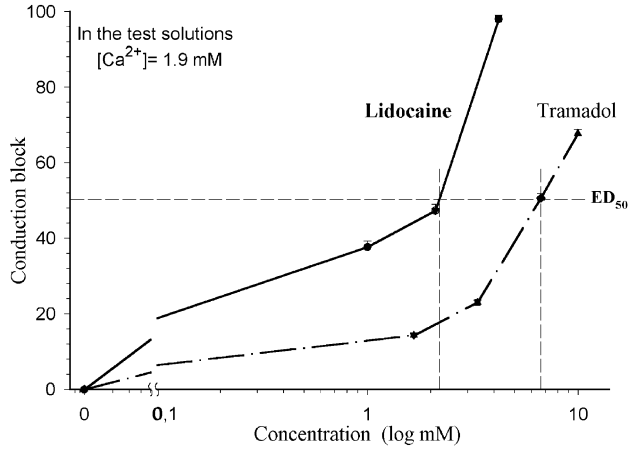

Fig. 3. Concentration dependence of compound action potential amplitude block (tonic conduction block) by externally applied different lidocane (●) and tramadol (▲) concentrations. All test solutions contain 1.9 mM Ca^{2+}. Ordinate: Tonic conduction block is expressed as the relative percentage decrease in amplitude (V) of compound action potential. Abscissa: Millimolar concentration of lidocaine and tramadol on log scale. Each point represents means ± S.E.M. ED_{50} values are 50% block produced by lidocaine and tramadol.

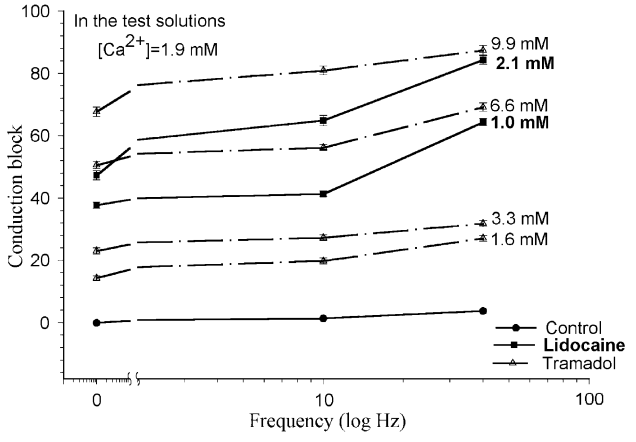

Fig. 4. Effects of conduction frequency on compound action potential amplitude block produced by different lidocaine (△) and tramadol (■) concentrations in Ringer's solution with 1.9 mM Ca^{2+}. Ordinate: Conduction blocks are expressed as percentages of relative decrease in the amplitude (V) of compound action potential. Abscissa: Stimulating frequency (Hz) or conduction frequency on log scale. In each trace, first data points show tonic conduction blocks (0 Hz); the other data points show phasic conduction blocks at 10 and 40 Hz frequencies. Each point represents the mean ± S.E.M.

3.1.2. Phasic conduction blocks

Phasic conduction blocks by tramadol and lidocaine were increased with a high conduction frequency (Fig. 4). The phasic block by lidocaine was however greater than that by tramadol. Although the tonic block by 6.6 mM tramadol (50.6%) was close to that by 2.1 mM lidocaine (47.3%), the phasic block by 6.6 mM tramadol (69.2%) was close to that by 1 mM lidocaine (64.4%). The three-fold difference in tonic blocks between tramadol and lidocaine concentrations was increased to around six-fold for phasic block.

3.2. Effect of Ca^{2+} on conduction blocks

The effects of Ringer's solutions, prepared with three different Ca^{2+} concentrations, are shown in Fig. 5. Values for solutions with 1.9 mM Ca^{2+} were taken as control. Tonic and phasic blocks were 7.8% and 12.8% in Ca^{2+} free Ringer's solution and 5% and 7.9% in 3.8 mM Ca^{2+} Ringer's solution, respectively.

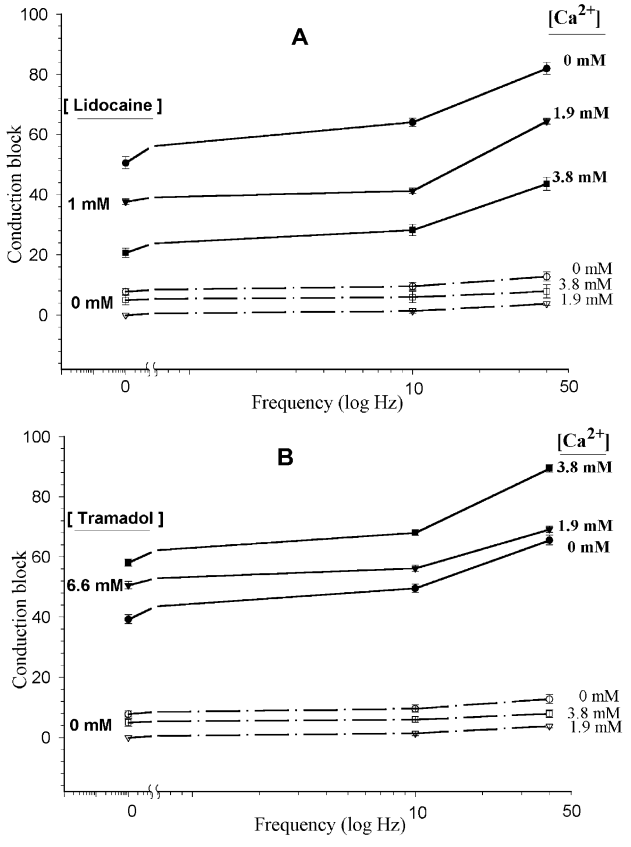

Fig. 5. Changes of conduction blocks by conduction frequency when Ca^{2+} free (0 mM) (○), 1.9 mM (▽) and 3.8 mM (□) Ca^{2+} concentrations in Ringer's solution either used alone (drug = 0 mM) (lower three lines in A and B) or in combination with 1 mM lidocaine (A) or 6.6 mM tramadol (B) Ordinates: Conduction blocks are expressed as the percentages of relative decrease in the amplitude (V) of compound action potential. Conduction blocks produced by drug-free Ca^{2+} solutions are used as control. Abscissa: Stimulating frequency (Hz) or conduction frequency on log scale. In each trace, first data points shows tonic conduction blocks; the other data points shows phasic conduction blocks. Each data point represents the mean ± S.E.M.

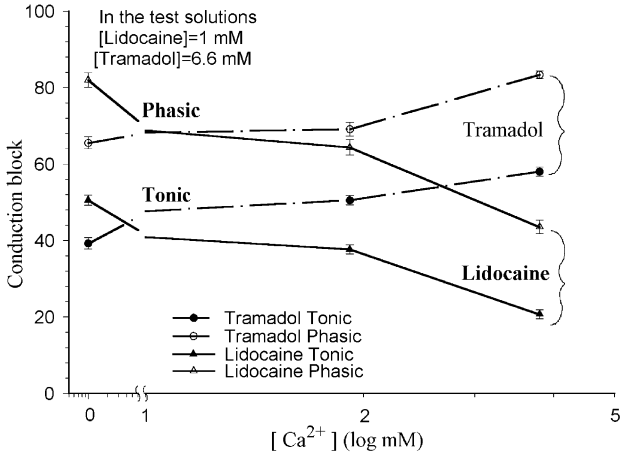

Fig. 6. Comparison of tonic and phasic conduction blocks produced by 6.6 mM tramadol (tonic ●, phasic ○) and 1 mM lidocaine (tonic ▲, phasic △). When Ca^{2+} concentration was increased in the test solutions, tonic and phasic blocks of lidocaine decreased, but both blocks by tramadol increased. Ordinate: Conduction blocks are expressed as percentages of relative decrease in the amplitude (V) of compound action potential. Abscissa: Millimolar Ca^{2+} concentrations of the test solutions on log scale. Each data point represents the mean ± S.E.M.

3.3. Effect of Ca^{2+} on blocks of tramadol and lidocaine

3.3.1. Tonic conduction blocks

In the presence of drugs, increasing the Ca^{2+} concentration of the test solution decreased the tonic block by lidocaine (Fig. 5A), but increased that by tramadol (Fig. 5B).

When the Ca^{2+} concentration was increased from 0 mM (free Ca^{2+}) to 3.8 mM, the 1 mM lidocaine tonic block decreased from 50.6% to 20.7%, but the 6.6 mM tramadol tonic block increased from 39.3% to 58.1% (Fig. 6).

3.3.2. Phasic conduction blocks

Raising the conduction frequency increased the blocks by tramadol and lidocaine (Fig. 4). Increasing the conduction frequency (0 → 40 Hz) and external Ca^{2+} concentration (0 → 3.8 mM) decreased the phasic block by lidocaine from 82% to 43.6%, but increased that by tramadol from 65.6% to 83.4% (Fig. 6).

4. Discussion

The figures show that increasing drug concentration and conduction frequency resulted in increased tonic and phasic blocks by tramadol and lidocaine. As shown in Figs. 3 and 4, the tonic and phasic blocking potencies of lidocaine were much stronger than those of tramadol.

Local anesthetics are known to produce blocks in addition to tonic block by increasing conduction frequency in

nerves (Courtney et al., 1978; Chernoff, 1990). In the present study, total block was produced after the additional block occurrences were taken as phasic block. The phasic block by lidocaine at high conduction frequencies could be explained according to the modulated receptor hypothesis (Hille, 1977): in the presence of lidocaine, increasing conduction frequencies enhances Na^+ channel inactivation and thus more lidocaine reaches to the binding site of Na^+ channels. Before the departure of lidocaine from the binding site as a response to subsequent stimuli, a relatively small number of Na^+ channels would open in the nerve membrane. As a result of the decreased number of Na^+ channels contributing to the occurrence of the compound action potential, the compound action potential amplitude gets smaller, with an increased conduction frequency.

The phasic block by tramadol as an opioid is similar to, but weaker, than that by lidocaine. This may imply that the latter mechanism applies to the block by tramadol. For better understanding of the mechanism involved in conduction blocks, the Ca^{2+} concentrations of the test solutions were changed. The results showed that the increase of Ca^{2+} concentration caused the lidocaine block to decrease, but that by tramadol to increase (Figs. 5 and 6).

The absence of Ca^{2+} from the test solutions reversed the results, that is as lidocaine block increases, the tramadol block decreases. It was reported earlier that free external Ca^{2+} causes an increase in membrane excitability by decreasing firing threshold, and an increase in inactivation of Na^+ channels by increasing their tendency to open state (Saito et al., 1984; Hille, 1992). In the absence of Ca^{2+} from the test solutions, the occurrence of lidocaine block could be explained by assuming that extension of inactivation in Na^+ channels allowed lidocaine to reach binding sites more readily, and thus the blocking activity of lidocaine increases. In addition, due to the increase in conduction frequency, additional block results from the combined effect. However, the tramadol block was decreased under the same experimental conditions.

For the drugs considered here, tramadol seems to have a blocking mechanism different from that of lidocaine. The fact that, in a patch clamp experiment, meperidine, an opioid, inhibits K^+ channels much more than Na^+ channels (Brau et al., 2000) encourages us to think that tramadol might have effects similar to those of meperidine.

The following conclusions could be drawn from the effects of tramadol and lidocaine, individually or combined with those of conduction frequency and Ca^{2+} concentration: (1) tramadol has a local anesthetic activity similar to but weaker than that of lidocaine; (2) tramadol may have a mechanism different from that of lidocaine for producing conduction blocks; (3) the presence of a high Ca^{2+} concentration in the external medium increases tramadol activity but decreases lidocaine activity.

In order to clarify the mechanism of tramadol action, further studies should be conducted with different bath solutions and recording conditions.

Acknowledgements

This work was supported by the Cukurova University Research Foundation Grant SBE 99.M.44.

References

Bokesh, P.M., Strichartz, G., 1986. Structure–activity relationship of lidocaine homologues producing tonic and frequency-dependent impulse blockade in nerve. J. Pharmacol. Exp. Ther. 237, 773–781.

Brau, M.E., Sander, F., Vogel, W., Hempelmann, G., 1997. Blocking mechanisms of ketamine and its enantiomers in enzymatically demyelinated peripheral nerves as revealed with single channel experiments. Anesthesiology 86, 394–404.

Brau, M.E., Koch, E.D., Vogel, W., Hempelmann, G., 2000. Tonic blocking action of meperidine on Na^+ and K^+ channels in amphibian peripheral nerves. Anaesthesiology 92, 147–155.

Chernoff, D.M., 1990. Kinetics analysis of phasic inhibition of neuronal sodium currents by lidocaine and bupivacaine. Biophys. J. 58, 53–68.

Courtney, K.R., Kendig, J.J., Cohen, E.N., 1978. Frequency-dependent conduction block: the role of nerve impulse pattern in local anesthetic potency. Anaesthesiology 48, 111–117.

Drachman, D., Strichartz, G.R., 1991. Potassium channel blockers potentiate impulse inhibition by local anaesthetics. Anaesthesiology 75, 1051–1061.

Hille, B., 1977. Local anaesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. J. Gen. Physiol. 69, 497–515.

Hille, B., 1992. Ionic Channels of Excitable Membranes, second ed. Sinauer Associates, Sunderland, MA, pp. 457–460.

Hunter, E.G., Frank, G.B., 1979. An opiate receptor on frog sciatic nerve axons. Can. J. Physiol. Pharmacol. 57, 1171–1174.

Marsh, S., 1989. Extracellular recording from rat sympathetic ganglia and whole nerve bundles: methods in experimental physiology and pharmacology. Biol. Meas. Tech. 6, 12–16.

Mert, T., Gunay, I., 1999. Comparison of frequency dependent blockade of procaine and lidocaine. Cukurova Univ. J. Health Sci. 14 (1,2,3), 7–12.

Pang, W.W., Huang, P.Y., Chang, D.P., Huang, M.H., 1999. The peripheral analgesic effect of tramadol in reducing propofol injection pain: a comparison with lidocaine. Reg. Anesth. Pain Med. 24 (3), 246–249.

Roux, P.J., Coetzee, J.F., 2000. Tramadol today. Curr. Opin. Anaesth. 13, 457–461.

Saito, H., Akutagava, T., Kitahata, L.M., Stagg, D., Collins, J.G., Scurlock, J.E., 1984. Interactions of lidocaine and calcium in blocking the compound action potential of frog sciatic nerve. Anaesthesiology 60, 205–208.

Stampfli, R., 1954. A new method for measuring potentials with external electrodes. Experientia 10, 508–509.

Strong, P.N., Smith, J.T., Keana, J.F.W., 1973. A convenient bioassay for detecting nanomolar concentrations of tetrodotoxin. Toxicon 11, 433–438.