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Do Adjuncts (Tramadol and Magnesium) Potentiate Impulse Inhibition by a Local Anesthetic in Isolated Frog Sciatic Nerves?

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Aim: In clinical use, the addition of adjuncts (magnesium or tramadol) to local anesthetic agents is used for different reasons. However, reports about the effect of magnesium plus bupivacaine and of tramadol plus bupivacaine on in vitro preparations are scarce. The aim of this study was to examine the ability of local anesthetics to reduce the amplitude of compound nerve action potentials of the isolated frog sciatic nerve in the absence and presence of adjuncts (magnesium and tramadol).

Materials and Methods: The experiments were done on frog sciatic nerves, using an extracellular recording technique. Isolated frog sciatic nerves were bathed in each test solution for 10 min. In each nerve, action potentials were recorded before exposure to the test solution, which served as the control data. The extracellular action potentials were recorded after 10 min of exposure to the test solution by using a BIOPAC MP 100 Acquisition System Version 3.5.7 (Santa Barbara, USA).

Results: Inhibition of the compound nerve action potentials induced by bupivacaine was markedly enhanced by magnesium but was not changed by tramadol. The amplitude of compound nerve action potentials increased from 15.10% with bupivacaine alone to 35.43% with bupivacaine plus magnesium.

Conclusions: These results suggest a potentially important interaction between magnesium and bupivacaine for nerve block, but there was no interaction between tramadol and bupivacaine.

Key Words: Bupivacaine, magnesium, tramadol, compound nerve action potential, frog sciatic nerve

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İzole Kurbağa Siyatik Sinirlerinin Lokal Anestezik Blokajı, Adjuvan Ajanlar (Tramadol ve Magnezyum) ile Güçlendirilebilir mi?

Amaç: Klinik uygulamalarda, farklı birçok nedenlerle lokal anesteziklere adjuvan ajanlar (magnezyum veya tramadol vb.) ilave edilmektedir. Ancak, magnezyum+bupivakain veya tramadol+bupivakain karışımlarının in vitro preparatlar üzerinde etkileri konusunda yapılan çalışmalar oldukça kısıtlıdır. Çalışmamızda in vitro olarak, bupivakain tramadol veya magnezyum ilavesinin bileşik sinir aksiyon potansiyeli üzerine etkisini araştırmayı planladık.

Yöntem ve Gereç: Deneyler, ekstrasellüler kayıt tekniği kullanılarak kurbağa siyatik sinirleri üzerinde yapılmıştır. İzole kurbağa siyatik sinirleri her bir test solüsyonunda 10 dakika süreyle banyo edilmiştir. Test solüsyonunda bekletmeden önce, her bir sinirden aksiyon potansiyelleri kaydedilmiş ve bu eğriler kontrol verileri olarak kabul edilmiştir. Ekstrasellüler aksiyon potansiyellerinin kaydedilmesi amacıyla BIOPAC MP 100 Veri Toplama Sistemi Version 3.5.7 (Santa Barbara, USA) kullanılmıştır.

Bulgular: Bileşik sinir aksiyon potansiyellerinin bupivakain ile inhibisyonu, ortama magnezyum eklenmesi sonucu belirgin bir biçimde artmış, fakat ortama tramadol eklenmesi sonuçlarda herhangi bir değişiklik oluşturmamıştır. Bupivakain içeren test solüsyonunda bileşik sinir aksiyon potansiyellerinin genliğinde gözlenen %15,10 luk inhibisyon, ortama magnezyum eklenmesi sonucu %35,43 olarak saptanmıştır.

Sonuç: Kullandığımız konsantrasyonlarda bupivakain magnezyum eklenmesinin sadece bupivakain kullanılması ile oluşturulan sinir bloku üzerinde artırıcı bir etkisi olduğu, fakat aynı etkinin bupivakain tramadol eklenmesi sonucu oluşmadığı kanısına varılmıştır.

Anahtar Sözcükler: Bupivakain, magnezyum, tramadol, bileşik sinir aksiyon potansiyeli, kurbağa siyatik siniri

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Introduction

Nerve cells are excitable, i.e., they are able to produce an action potential after the application of suitable stimulus. Action potentials are rapid changes in cell membrane potential from the “resting” or depolarized state. Measurement of action potential amplitude may provide information about membrane Na⁺ transport. Compound nerve action potential (CNAP) amplitude is positively correlated with sodium transport. In addition, the action potential amplitude recorded from nerves can be used to estimate the number of activated nerve fibrils (1).

Local anesthetics block the propagation of nerve impulses by binding to receptors on the sodium channel and preventing normal function (2,3,4). Bupivacaine hydrochloride (amide local anesthetic) is the most frequently used local anesthetic for operative and postoperative pain relief in many countries.

Adjuncts to local anesthetics for regional anesthesia may enhance the quality and duration of block. Opioids and magnesium are such examples (5,6,7,8,9). Tramadol hydrochloride is a centrally acting analgesic that acts at opioid receptors on excitable cell membranes and also appears to modify transmission of pain impulses by inhibition of monoamine reuptake (10). It was indicated that drug activation of opioid receptors inhibits action potential production (11). It is postulated that tramadol has a local anesthetic effect similar to that of lidocaine (amide local anesthetic, such as bupivacaine) (12,13).

Magnesium, one of the most plentiful divalent cations of the body, plays an important role in many of the functions of the nervous system. For example, magnesium elevates the firing threshold of the nerve action potential (14). Magnesium also reduces the acetylcholine release from nerve terminals at the neuromuscular junction (15).

In clinical use, the addition of adjuncts (magnesium or tramadol) to local anesthetic agents is used for different reasons (5,6,7,8,9). However, the effect of magnesium plus bupivacaine and of tramadol plus bupivacaine on in vitro preparations has not been studied thus far.

In the present study, we have examined the interaction of tramadol plus bupivacaine on CNAP in an isolated frog myelinated sciatic nerve and compared these effects with those of magnesium plus bupivacaine. Our purpose in conducting these studies was to determine whether the addition of magnesium or tramadol to bupivacaine could enhance local anesthetic nerve block.

Materials and Methods

The study design was approved by the Ethics Committee of the Faculty of Medicine, University of Mersin. Animals were used throughout the experiments according to the proposals of the US National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Tissue preparation

Rana cameroni frogs weighing 30-40 g were used in the experiments. The sciatic nerves were excised from rapidly decapitated and pithed frogs and maintained in normal frog Ringer’s solution. This solution was composed of 115 mM NaCl, 2.5 mM KCl, 2 mM CaCl₂ and 2.38 mM NaHCO₃. The pH of the frog Ringer’s solution was adjusted to 7.2 and all measurements were recorded with the preparations equilibrated at room temperature (21-23 °C).

The osmolarity of frog Ringer’s solutions with magnesium were maintained at approximately 295 mOsm with the addition of sucrose. This insured that effects seen with addition of magnesium were not due to variations in osmolarity.

Test solutions

After control studies in frog Ringer’s solution, as seen in Table 1, each of the nerves was bathed with bupivacaine (Group B), tramadol (Group T), a calcium-free normal frog Ringer’s solution containing magnesium (Group M), and bupivacaine with tramadol (Group BT) or magnesium (Group BM). Deionized and bi-distilled water was used for the solutions. The pH of the solutions was adjusted to 7.2. After control studies in frog Ringer’s solution for each nerve preparation, only one test solution was studied in each nerve preparation. The bupivacaine hydrochloride powder used in this experiment was purchased from Sigma Chemical Co., St. Louis, USA (B-5274). Tramadol hydrochloride powder was supplied by Abdi Ibrahim Pharmaceutical Company, Istanbul, TURKEY.

Table 1. Drug concentrations (mM) used for the test solutions.

Test Solutions	Concentration for single drug or combinations of drugs (mM)
Group B = bupivacaine	2
Group M = magnesium	10
Group T = tramadol	8
Group BM = bupivacaine+magnesium	2 +10
Group BT = bupivacaine+tramadol	2 + 8

Electrophysiological techniques

The experiments were carried out in vitro using extracellular recording techniques (16). After 30 min of stabilization in frog Ringer's solution, segments of nerves measuring 4-5 cm were placed in a 5 cm x 15 cm Plexiglas nerve chamber containing Ag/AgCl electrodes. The space between the electrodes was 0.5 cm. The nerves were stimulated with these electrodes, at a voltage set to produce a maximal CNAP using single square pulses of supramaximal strength and 0.5 ms in duration.

Experimental protocols

After CNAP had stabilized in frog Ringer's solution, CNAPs were recorded using a BIOPAC MP100 Acquisition System Version 3.5.7 (Santa Barbara, USA) from each nerve before exposure to each test solution and these data were accepted as the control. In this design, each subject served as its own control. Then each nerve was bathed for 10 min in the test solution. After the 10 min drug exposure, CNAPs were recorded and these data were accepted as the experimental group data.

Statistical Analysis

Normalized CNAP values for changes with all test solutions were reported as percentages of control amplitude (mean \pm SE). The same subject groups were observed twice (control and experimental group); therefore, each subject served as its own control. Paired *t*-test was used to compare the difference between the control and experimental groups. In addition, one-way ANOVA was used to compare the difference between groups concerning the degree of block. After ANOVA test, Tukey test as a post-hoc test was used for significant differences. In the statistical analysis, maximum Type I error rate was accepted as 0.05.

During the experiments, the conduction blocks produced by test solutions on CNAP amplitude (V) were calculated using the following formula:

$$\text{Degree of Block} = [(V_{\text{Control}} - V_{\text{Experiment}}) / V_{\text{Control}}] \times 100 \quad \text{Equation A}$$

Thus, the conduction blocks were defined as the percentage of relative decrease in the amplitude of CNAP.

Effects of Magnesium, Tramadol and Bupivacaine Alone and of Bupivacaine Combinations with Magnesium or Tramadol on the CNAP

The effect of 10 mM magnesium on CNAP amplitude was measured in frog Ringer's magnesium solution in which calcium was replaced by 10 mM magnesium. The osmolarity of frog Ringer's solutions with magnesium was maintained at approximately 295 mOsm with the addition of sucrose. This insured that effects recorded during the addition of magnesium were not due to variations in osmolarity. The effects of bupivacaine (2 mM) and tramadol (8 mM) on CNAP amplitude were also measured in frog Ringer's solution. These amplitude findings were compared with those produced in normal frog Ringer's solution. Table 2 shows the mean amplitude values of bupivacaine, tramadol and magnesium administrations alone and of bupivacaine combined with tramadol and magnesium.

Table 2. Effects of bupivacaine and/or adjuncts (tramadol and magnesium) on compound nerve action potentials (CNAPs). There was no statistically significant difference between the control mean amplitudes of any group.

	Group B	Amplitude [#] (mV)
Control		2.10 \pm 0.05
Experiment		1.78 \pm 0.06*
	Group M	
Control		2.15 \pm 0.07
Experiment		1.93 \pm 0.06*
	Group T	
Control		2.06 \pm 0.06
Experiment		1.84 \pm 0.05*
	Group BM	
Control		2.06 \pm 0.05
Experiment		1.69 \pm 0.04*
	Group BT	
Control		2.07 \pm 0.05
Experiment		1.81 \pm 0.04*

[#] Amplitude values are mean \pm SE; n =10 for each group.

* P < 0.05, each drug compared with its own control

The results of magnesium, tramadol and bupivacaine extracellular administrations alone and of bupivacaine combined with others on sciatic nerve action potentials are shown in Figure 1.

As seen in Table 2 and Figure 1, the amplitude of action potential was decreased in all experimental groups. There were significant differences ($p = 0.0001$) among the controls and the experiments regarding these CNAP amplitudes for bupivacaine, tramadol and magnesium alone and bupivacaine combined. However, the only significant amplitude difference among the experimental groups was between bupivacaine combined with magnesium and the others. As seen in Table 3 and Figure 2, the degrees of block for tramadol, magnesium and bupivacaine were 10.16%, 10.29% and 15.35%, respectively, according to the formula given in Equation A.

In the presence of bupivacaine, increasing Mg^{2+} concentration of the test solution from 0 mM (free Mg^{2+}) to 10 mM produced visible change in the degree of block by bupivacaine (Table 3 and Figure 2). When the Mg^{2+} concentration was increased from 0 mM (free Mg^{2+}) to 10 mM, degree of block with bupivacaine increased from

15.35% to 35.17%. However, adding tramadol to bupivacaine did not produce as significant a change in the degree of block by bupivacaine (Table 3 and Figure 2).

Discussion

The present study was designed to examine the effects of magnesium and tramadol on the nerve block produced by bupivacaine. The experiments were done on frog myelinated sciatic nerves. Although the frog myelinated sciatic nerve is not a mammalian nerve, it has physiologic and morphologic properties much like those of mammalian peripheral nerves (17,18) and responds to blocking doses of local anesthetics in a way that is comparable to its mammalian counterpart (19,20).

Our results confirm that the amplitude of CNAP recorded in the frog sciatic nerve is diminished by 10-min exposure to all test solutions (see Table 1 and Figure 1). This inhibition for bupivacaine was due to a blockage of the propagation of nerve impulses by binding to receptors on the sodium channel and preventing normal function (3).

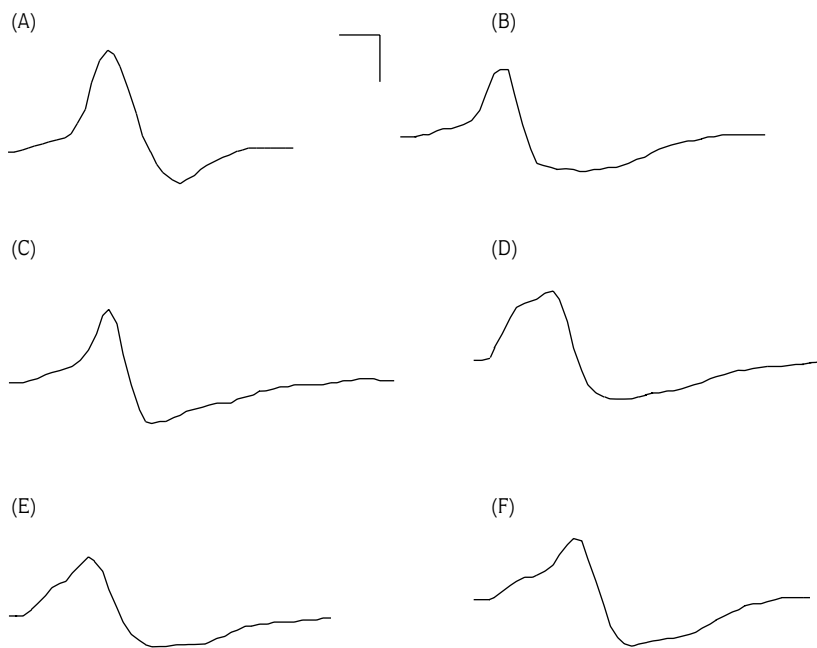


Figure 1. The effects of extracellular magnesium, tramadol and bupivacaine alone or bupivacaine combined with the other two on sciatic nerve action potentials. CNAP records of control (A), after bupivacaine (B), magnesium (C), and tramadol (D), and after bupivacaine with magnesium (E) and bupivacaine with tramadol (F). Calibrations for all traces are shown in A; vertical bar = 0.8 mV; horizontal bar = 0.07 ms.

Table 3. The degree of block for groups as calculated using the formula given in Equation A, demonstrating the percentage of relative decrease in the amplitude of CNAP.

Groups	Drug Concentration (mM)	Degree of Block (%)
Group B	2	15.35 ± 1.26
Group M	10	10.29 ± 1.14
Group T	8	10.16 ± 0.88
Group BM	2 + 10	^a 35.17 ± 2.92
Group BT	2 + 8	14.39 ± 1.08

Values are mean ± SE; n = 10.

^aP < 0.01, Group BM compared with the other groups

Group B = bupivacaine.

Group M = magnesium.

Group T = tramadol.

Group BM = bupivacaine+magnesium.

Group BT = bupivacaine+tramadol.

Tramadol has been proven to exert a local anesthetic-type effect on peripheral nerves in both clinical and laboratory studies (21,22). Tsai et al. (5) studied the effects of tramadol on the sciatic nerve with somatosensory evoked potentials (SSEPs). They showed that tramadol could block neural conduction through mechanisms that are separate from opioid receptors. Brau et al. (23) concluded that meperidine (an opioid) has a nonselective inhibitory action on Na⁺ and K⁺ channels of the amphibian peripheral nerve. For tonic Na⁺ channel block, neither an opioid receptor nor the local anesthetic agent-binding site is the target site for meperidine block. Thus, it could be said that as with meperidine, the action site of tramadol is probably not the local anesthetic agent-binding site and tramadol may have a mechanism different from that of bupivacaine for producing conduction blocks. In a randomized, double-blinded study by Kapral et al. (6), addition of tramadol to 1% mepivacaine for axillary brachial plexus block resulted in a significant increase in the duration of the blockade without any side effect. In our study, the significant decrement in the amplitude of the CNAP in tramadol is probably due to local anesthetic-like action of this drug. If tramadol acts like a local anesthetic, binding to the same receptor sites and inhibiting nerve conduction, then drugs

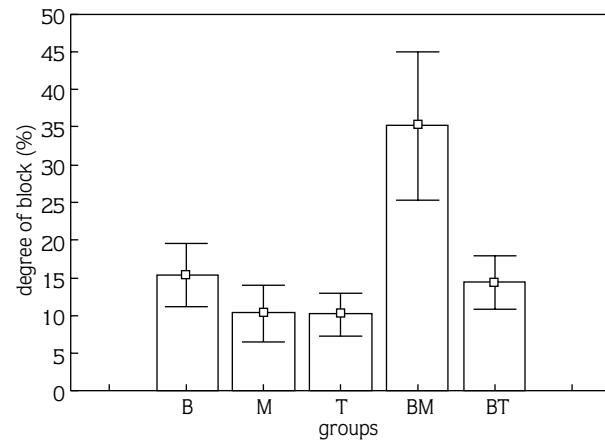


Figure 2. The degree of block for groups as calculated using the formula given in Equation A, demonstrating the percentage of relative decrease in the amplitude of CNAP. Note that bupivacaine plus magnesium caused a significant ($P < 0.01$) increase in the bupivacaine-induced conduction block. Group B = bupivacaine, group M = magnesium, group T = tramadol, group BM = bupivacaine+magnesium, group BT = bupivacaine+tramadol.

that alter the action of local anesthetics may have a similar effect on it. However, in this present study, it has been demonstrated that addition of tramadol to bupivacaine did not produce any changes in nerve block by bupivacaine. The observed interactions of bupivacaine and tramadol suggest that the drugs either have different binding sites or different action mechanisms. Similarly, Mert et al. (22) have shown a definitive local anesthetic effect of tramadol in experiments on frog sciatic nerves. In their animal study, adding calcium to the test solution decreased the local anesthetic effect of lidocaine but enhanced that of tramadol. Therefore, the blocking mechanism of the two drugs is likely to be different.

Magnesium ions are known to elevate the firing threshold in both myelinated and unmyelinated axons (24). It has been suggested that divalent cations reduce the fixed negative surface charge on the outside nerve membrane and, in so doing, increase the transmembrane potential, i.e., cause a hyperpolarization (25,26,27). If the fiber is hyperpolarized, it is more difficult for it to reach threshold level and thus conduction block will occur. In present study, it has been demonstrated that addition of magnesium to bupivacaine can result in an enhancement of nerve block by bupivacaine. The increased conduction block in the presence of magnesium

may have been due to an increased number of fibers failing to reach threshold and thus failing to fire. Similar findings were reported for lidocaine (amine local anesthetic), benzocaine (neutral local anesthetic), and QX 572 (quaternary derivative of lidocaine) (28). Akutagawa et al. (28) measured compound action potentials in whole sciatic nerves from frogs and found that increased magnesium concentrations enhanced conduction blocks produced by lidocaine, benzocaine and QX 572. Our result

that addition of magnesium enhanced the nerve block by bupivacaine can be explained by an effect on the nerve membrane.

In conclusion, better conduction block was observed with magnesium addition to bupivacaine but not with tramadol addition to bupivacaine. Further research with different bath solutions and recording conditions is needed to support relationships between bupivacaine, tramadol and magnesium.

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