Polish Journal of Pharmacology
Pol. J. Pharmacol., 2003, 55, 25–30
ISSN 1230-6002

EFFECTS OF CALCIUM AND MAGNESIUM ON PERIPHERAL NERVE CONDUCTION

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Effects of calcium and magnesium on peripheral nerve conduction. T. MERT, Y. GUNES, M. GUVEN, I. GUNAY, D. OZCENGIZ. Pol. J. Pharmacol., 2003, 55, 25–30.

Divalent cations, such as calcium and magnesium, are constantly present in extracellular compartment of most organisms. Modification of extracellular concentrations of divalent ions causes changes in physiologic functions, such as excitability and conduction of the nerves. The present study was designed to investigate and compare the effects of calcium and magnesium on nerve conduction and lidocaine-induced nerve conduction block. The aim of our study was to contribute to better understanding of physiological and pharmacological roles of divalent cations.

Experiments were conducted on the sciatic nerves by using the sucrose-gap recording technique. We evaluated the effects of test solutions containing different calcium or magnesium concentrations, prepared with or without lidocaine, on compound action potentials to determine physiological and pharmacological roles of these cations. After the control recordings, the nerve was exposed to Ringer's solution containing 0, 1.9, 3.8 mM Ca^{2+} and 1.9 and 3.8 mM Mg^{2+} with or without 1 mM lidocaine. Decreasing the Ca^{2+} concentrations in Ringer's solution with or without lidocaine enhanced both tonic and phasic blocks. However, increased Mg^{2+} concentration did not change the tonic blocks but increased the phasic blocks.

In conclusion, the results suggested but not prove that Ca^{2+} and Mg^{2+} may have different mechanisms of action on peripheral nerves. While Ca^{2+} directly affects the gating of Na^{+} channels, action of Mg^{2+} can be explained by surface charge theory.

Key words: *divalent ions, calcium, magnesium, lidocaine, nerve conduction, frog sciatic nerve*

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INTRODUCTION

The conduction of action potential in a nerve most frequently requires the coordinated opening and closing of ion channels that participated in action potential electrogenesis [1, 5]. It is well known that Na^+ current is responsible for depolarization, and K^+ current accounts for repolarization of the spike in the formation of the nerve action potential [13, 19].

Under normal conditions, most organisms maintain constant extracellular concentrations of divalent ions. The extracellular compartment is the basic source of all intracellular ions [12, 14]. In some previous studies, it has been demonstrated that the modification of extracellular concentrations of divalent ions changes the nerve conduction [16]. Divalent cations are known to have strong effect of the gating properties of voltage-dependent ion channels [3, 14]. These effects of divalent cations on gating kinetics of Na^+ channels are usually explained by surface charge theory. This hypothesis holds that divalent cations alter the gating by neutralizing negative charge at the membrane surface, thus changing the local field near the voltage-sensing parts of the channels [12, 14, 18]. In some previous researches, interactions between divalent ions and local anesthetics have been studied, and it was reported that the conduction blocks induced by local anesthetics were changed by the modification of divalent cations concentration in the external medium [16, 18]. However, mechanism of action of those agents has not been explained in detail. It is well known that local anesthetics, such as lidocaine, block the nerve conduction in a concentration-dependent manner by interfering with the regenerative increase in Na^+ permeability in peripheral nerves [4, 7, 8, 15]. Development of this block is mediated by binding to specific binding sites on voltage-dependent Na^+ channels. Activity of local anesthetics depends on the conformation or state of the Na^+ channels [17]. Since they bind more efficiently to open and inactivated Na^+ channels than to those in the resting state, action of local anesthetics on the nerve conduction is enhanced by increasing the stimulation frequency [6, 9–11].

Our objective in this study was to investigate the mechanisms of action of Ca^{2+} and Mg^{2+} on nerve conduction which are important for understanding of physiological processes, such as nerve excitability and function of neuromuscular junctions. We also attempted to explain the interactions of these cations with lidocaine that is a local anesthetic.

MATERIALS and METHODS

Preparation

Electrophysiological experiments were conducted on frogs (70–80 g). Frogs (*Rana cameranoi*) were rapidly decapitated, and then the sciatic nerves were dissected from the lumbar plexus to the knee. The nerves, if not used immediately, were kept in Ringer's solution referred to as normal electrolyte solution at 4–6°C for a day. Ethics Committee of Cukurova University Medical Sciences Research Center approved this study.

Stimulation and recording instruments

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 2 channels, Master flex perfusion pump with 8 channels and A/D card + personal computer were used in the experiments.

Drugs and 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*: Ringer's solutions free of or supplemented with divalent cations at 1.9 or 3.8 mM Ca^{2+} or Mg^{2+} without lidocaine or with 1 mM lidocaine.

Deionized and redistilled water was used to prepare the solutions. All solutions were bubbled with 95% O_2 and 5% CO_2 gas mixture. The pH values of all solutions were adjusted to 7.4 by using NaOH or HCl, if needed.

Electrophysiological measurements

In this study, we have used the sucrose-gap recording technique that allows for measurement of monophasic compound action potential with extracellular agar-bridged Ag-AgCl electrodes [20, 21]. The experimental set-up has been previously described by Mert et al. [15]. Briefly, after the nerves were desheated (removal of circumferential sheath), the nerves were positioned across a modified sucrose-gap apparatus (Fig. 1A) partitioned into com-

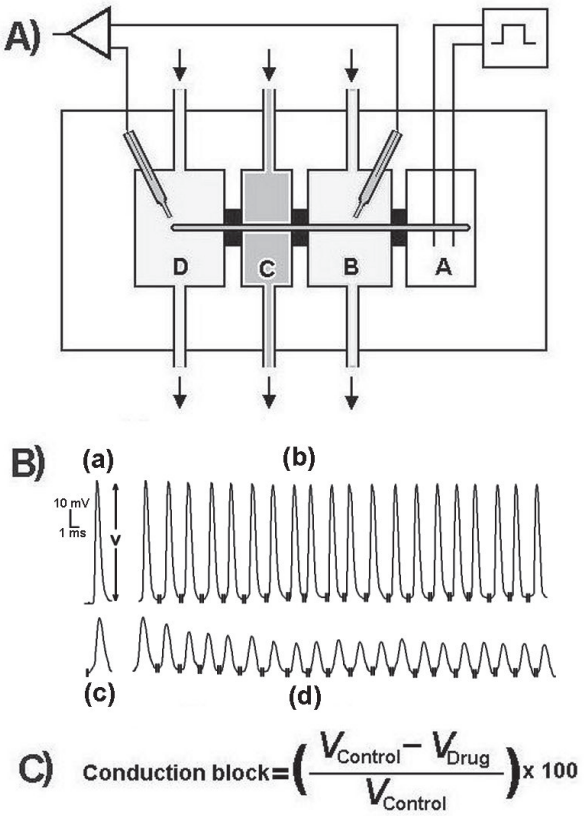

Fig. 1. **A**) Sucrose-gap apparatus: Pool A, containing a pair of stimulating platinum electrodes, is filled with mineral oil to protect nerves from drying; Pool B contains Ringer's or test solution; Pool C contains isotonic sucrose and Pool D contains isotonic KCl solution. The potential difference between pool B and D was recorded by using agar bridged Ag-AgCl electrodes. All solutions were perfused at the rate of 2–3 ml per minute. **B**) Example of tonic and phasic conduction blocks by 1 mM lidocaine in Ringer's solution without divalent cations. The control (in normal Ringer's solution) responses (**a**) to a single stimulus (tonic response) (**b**) to a 40 Hz train lasting 500 ms. (**c**) and (**d**) tonic and phasic responses, respectively, 35 min after the drug application. **C**) The formula used to calculate nerve conduction block. Tonic block, which 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

partments by vaseline-slicone oil mixture, for stimulation and recording. Before starting the experiment, the nerve was superfused with Ringer's solution in order to achieve stable baseline and reproducible compound action potentials. The nerves were stimulated supramaximally (1.5 to 2 times of maximal intensity) with 0.05 ms duration square-wave voltage pulses. After the control values were recorded, the nerve was subjected to tonic stimulation by a single stimulus or to phasic stimulation by repetitive stimuli at 10 Hz train, lasting 1000 ms and 40 Hz train, lasting 500 ms. Then, the preparation was exposed to the drug to be tested over a period of 35 min. The nerve was stimulated once per 5 min (tonic stimulation) and the responses were recorded for 35 min. At the end of this period, the recorded compound action potential was accepted as a frequency-independent response (tonic response). Frequency-dependent responses (phasic response) were recorded, according to the protocol mentioned above (Fig. 1B). Then all recordings were transferred to the computer in order to measure response characteristics and to evaluate them. Test solutions were applied when the nerves had control compound action potential amplitude of over 35 mV. All experiments were carried out at room temperature (21–23°C).

Statistical analysis

The changes in normalized compound action potential values induced by all test solutions were reported as percentage of control amplitude (mean ± SE). The differences due to the application of test solutions were tested for significance with Mann-Whitney *U*-test. Significance was set at $p < 0.05$.

RESULTS

In the experiments, the magnitude of block of the compound action potential amplitude produced by test solutions was quantified using the formula shown in Figure 1C. Evaluation of phasic blocks is very important. It has been reported that a reduction in Na^+ conductance is the basis for phasic block. Because nerves signal by repeated impulses *in vivo*, it is important to explain the effect of stimulation frequency on drug-induced nerve blocks [15, 16].

Compound action potentials were recorded in Ringer's solution free of divalent cations (n = 9) or supplemented with 1.9 (normal Ringer's solution) (n = 8) or 3.8 mM Ca^{2+} (n = 8) and 1.9 (n = 6) or 3.8 mM Mg^{2+} (n = 6) (Fig. 2). Compound action potentials recorded in Ringer's solution with 1.9 mM Ca^{2+} (normal Ringer's solution) were taken as the control. In normal Ringer's solution, tonic block did not appear, while phasic block was 3.2 ± 0.5% at 40 Hz.

In the presence of Ringer's solution free of divalent cations, tonic block was 7.1 ± 0.7%, while phasic block was 12.2 ± 0.9% at 40 Hz. When Ca^{2+} concentration was raised to 3.8 mM, tonic and pha-

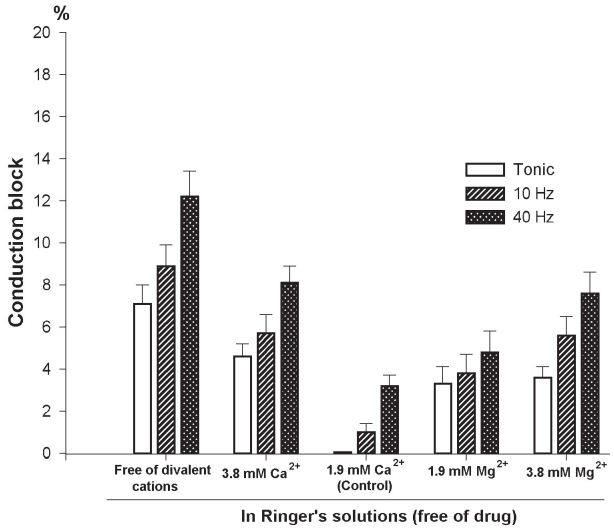

Fig. 2. Changes in conduction blocks induced by different stimulating frequencies when the frog sciatic nerve was exposed to Ringer's solution prepared without divalent cations and suplemented with 1.9, 3.8 mM Ca^{2+} or 1.9, 3.8 mM Mg^{2+}. Ordinates: conduction blocks are expressed as the percentages of relative decrease in the amplitude (V) of compound action potential according to the equation shown in Figure 1. Conduction blocks produced by Ringer's solution with 1.9 mM Ca^{2+} are used as the control. Abscissa: Ca^{2+} and Mg^{2+} concentrations (mM) in Ringer's solutions. Each data point represents the mean ± SE

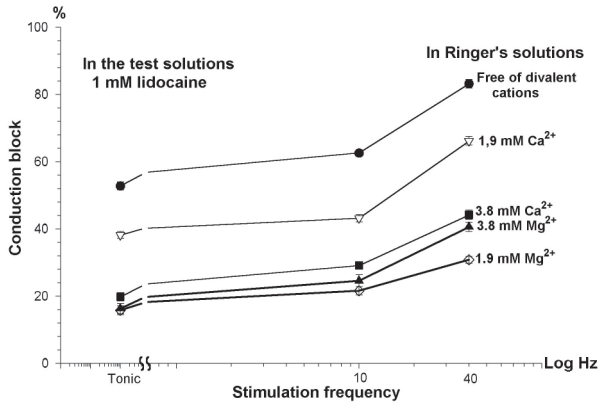

Fig. 3. Effects of conduction frequency on compound action potential amplitude block produced by 1 mM lidocaine in Ringer's solution without divalent cations (●) and supplemented with 1.9 (▽), 3.8 (■) mM Ca^{2+} or 1.9 (◇), 3.8 (▲) mM Mg^{2+}. Ordinate: conduction blocks are expressed as percentages of relative decrease in the amplitude (V) of compound action potential according to the equation shown in Figure 1. 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 Hz and 40 Hz. Each point represents the mean ± SE

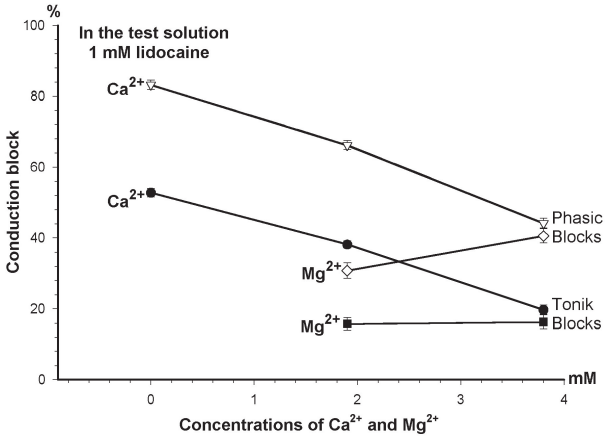

Fig. 4. Comparative effects of Ca^{2+} (tonic ●, phasic ▽) and Mg^{2+} (tonic ■, phasic ◇) on tonic and phasic conduction blocks induced by 1 mM lidocaine. When Ca^{2+} concentration was increased in the test solutions, tonic and phasic blocks of lidocaine decreased. However, both blocks increased when Mg^{2+} concentration was increased. Ordinate: conduction blocks are expressed as percentages of relative decrease in the amplitude (V) of compound action potential according to the equation shown in Figure 1. Abscissa: Ca^{2+} and Mg^{2+} concentrations (mM) in the test solutions. Each data point represents the mean ± SE

sic blocks decreased to 4.6 ± 0.6% and 8.1 ± 0.8%, respectively.

To determine the Mg^{2+} effects on nerve conduction, Ca^{2+} in Ringer's solution was replaced by Mg^{2+} at the same concentration. Increasing the Mg^{2+} concentration from 1.9 to 3.8 mM did not cause any statistically significant difference ($p > 0.05$) in tonic conduction blocks (Fig. 2). However, there were significant differences ($p < 0.05$) between mean values in phasic blocks (4.8 ± 1.0% and 7.6 ± 1.0%, respectively).

Our previous study [15] demonstrated that blocking action of lidocaine was enhanced when lidocaine concentration or stimulation frequency increased. In the presence of 1 mM lidocaine in Ringer's solution free of divalent cations (n = 9), tonic block was 52.8 ± 1.0% and phasic block was 83.2 ± 1.1% at 40 Hz. Raising the Ca^{2+} concentration from 1.9 to 3.8 mM decreased the tonic conduction blocks from 38.2 ± 0.8% to 19.8 ± 1.0% and phasic conduction blocks from 66.2 ± 1.1% to 44.2 ± 1.4%, respectively (Fig. 3). In the presence of 1 mM lidocaine, when Mg^{2+} concentration in Ringer's solution increased from 1.9 to 3.8 mM, tonic blocks rose from 15.8 ± 1.0% to 16.3 ± 1.7% and phasic blocks was enhanced from 30.8 ± 1.7% to 40.6 ± 1.5%, respectively (Fig. 4). The tonic blocks did

not change statistically significantly ($p > 0.05$) in the presence of different Mg^{2+} concentrations in the test solution.

DISCUSSION

Effects of calcium

Calcium and some other divalent cations have well-documented effects on the properties of Na^+ channels [12]. The effects of Ca^{2+} on gating kinetics and open probability are usually explained by the surface charge hypothesis [12, 16]. For the action of Ca^{2+} ions on gating properties of Na^+ channels, Armstrong suggested instead that channels occupancy by Ca^{2+} is the basis for these effects [2, 3]. According to this hypothesis, Na^+ channels can close while blocked or occupied by Ca^{2+}. Ca^{2+} ions transiently block Na^+ channels, which shortens the time course for closing of their activation gates.

Absence of divalent cations from the test solution causes an increase in the number of inactivated Na^+ channels by increasing their tendency to open which produces a partial conduction block in a nerve. In addition, an increase in the stimulation frequency further enhances the number of inactivated channels [9, 11, 15]. Because of the decreased number of active Na^+ channels contributing to the occurrence of the compound action potential, conduction block is enhanced with an increased stimulation frequency. When external Ca^{2+} concentration is increased, Ca^{2+} occupancy of Na^+ channels raises, and stabilizes the closed state of these channels.

For better understanding of the mechanism of Ca^{2+} action involved in development of conduction block, the test solutions were supplemented with lidocaine. The results showed that an increase in Ca^{2+} concentration caused a decrease in lidocaine blocks. Lidocaine applied with normal Ringer's solution (with 1.9 mM Ca^{2+}) blocked the nerve conduction and produced additional block at high stimulation frequencies. Blocking action of lidocaine can be explained by modulated receptor hypothesis [6, 9, 12, 15]. According to this hypothesis, after lidocaine molecules access the axoplasm by hydrophilic pathway, they reach binding sites on Na^+ channels and block them. Increasing the conduction frequency enhances Na^+ channel inactivation and thus more lidocaine reaches the binding site and more Na^+ channels are blocked by lidocaine molecules.

The absence of Ca^{2+} from the test solutions containing lidocaine enhanced the lidocaine-induced tonic and phasic block in comparison with normal Ringer's solution. It is known that lidocaine binds more effectively to the open and inactivated Na^+ channels than to those in the resting states [6, 9]. The absence of Ca^{2+} causes an increase in the number of open and inactivated Na^+ channels. Therefore, more lidocaine may reach the binding sites more readily, and its blocking activity may increase. In addition, due to the increase in stimulation frequency, additional block may result from the combined effect.

An increase in Ca^{2+} concentration (from 1.9 to 3.8 mM) decreased the lidocaine-induced blocks. It can be explained by the fact that high Ca^{2+} concentrations have a distinct blocking action on Na^+ current though open Na^+ channels. Ca^{2+} appears to stabilize the resting state, making the channels less likely to open [3, 12]. Therefore, less lidocaine molecules may bind to the binding site and blocking action of lidocaine may decrease.

The other possible explanation is that Ca^{2+}-activated K^+ channels may be activated by increasing the concentration of Ca^{2+} inside the cell [13]. It is known that during the action potential, Ca^{2+} ions enter the axoplasm through Na^+ channels and activate the Ca^{2+}-activated K^+ channels [2, 3, 13]. These channels repolarize the action potential and accelerate the closing of Na^+ channels. So, when extracellular Ca^{2+} concentration increases, blocking action of lidocaine on Na^+ channels decreases.

Effects of magnesium

The effects of Mg^{2+} on nerve conduction were measured in Ringer's solution in which Ca^{2+} was replaced by Mg^{2+} at the same concentrations. The results presented in this paper suggested that action of Mg^{2+} could be explained by the surface charge theory [12, 14]. This theory suggests that external surface of membrane bears a negative net charge. Mg^{2+} is attracted by these charges on membrane surface. Therefore, Mg^{2+} changes gating of Na^+ channels by neutralizing negative charge, thus altering the local field near the voltage-sensing parts of the channels. Increasing the transmembrane potential could cause a hyperpolarization. If the nerve fiber is hyperpolarized, it is more difficult for it to reach threshold level and thus conduction block will occur.

When Mg^{2+} concentration was enhanced from 1.9 to 3.8 mM, tonic block was not changed, but phasic block was enhanced. In the same way, raising the Mg^{2+} concentration only changed the phasic lidocaine-induced block.

These results strongly suggest that an increase in Mg^{2+} concentration does not affect the nerve conduction in resting state (during tonic stimulation). However, the increase in phasic block (with or without lidocaine) in the presence of high Mg^{2+} concentration indicates that Mg^{2+} can slow the closing of Na^+ channels and enhance the number of inactivated Na^+ channels. Therefore, more lidocaine can reach the binding site on Na^+ channels and block more Na^+ channels.

In conclusion, the results presented in this paper suggest, but not prove, that extracellular Ca^{2+} may close the Na^+ channels. An increase of the extracellular Ca^{2+} concentration may reduce the blocking potency of lidocaine. Ca^{2+} entry through Na^+ channels may also cause an increase in activity of the Ca^{2+}-activated K^+ channels [13]. However, effects of Mg^{2+} are different from Ca^{2+}. Actions of Mg^{2+} can be explained by surface charge theory. In addition, an increase in the Mg^{2+} concentration enhances the potency of lidocaine at high stimulation frequency.

Acknowledgment. This work was supported by the Cukurova University Research Foundation Grant TF2002BAP33.

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Received: September 19, 2002; in revised form: January 6, 2003.