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SCIENTIFIC ARTICLE

Influence of different local anesthetics on atracurium neuromuscular blockade on rats☆☆

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Animals;
Rats

Abstract
Introduction: The association between local anesthetics (LA) and neuromuscular blocking (NMB) drugs in clinical practice, and the possibility of interaction between these drugs has been investigated. LAs act on neuromuscular transmission in a dose-dependent manner and may potentiate the effects of NMB drugs.
Objective: The aim of this study was to evaluate, in an experimental model, the effect of lidocaine and racemic bupivacaine on neuromuscular transmission and the influence on neuromuscular blockade produced by atracurium.
Methods: Male Wistar rats, weighing from 250 to 300 g were used. The preparation was set up based on a technique proposed by Bülbring. Groups were formed (n = 5) according to the drug studied: lidocaine 20 $\mu g.mL^{-1}$ (Group I); racemic bupivacaine 5 $\mu g.mL^{-1}$ (Group II); atracurium 20 $\mu g.mL^{-1}$ (Group III); atracurium 20 $\mu g.mL^{-1}$ in a preparation previously exposed to lidocaine 20 $\mu g.mL^{-1}$ and racemic bupivacaine 5 $\mu g.mL^{-1}$, Groups IV and V, respectively. The following parameters were assessed: 1) Amplitude of hemi diaphragmatic response to indirect stimulation before and 60 minutes after addition of the drugs; 2) Membrane potentials (MP) and miniature endplate potentials (MEPPs).
Results: Lidocaine and racemic bupivacaine alone did not alter the amplitude of muscle response. With previous use of lidocaine and racemic bupivacaine, the neuromuscular blockade (%) induced by atracurium was 86.66 ± 12.48 and 100, respectively, with a significant difference ($p = 0.003$), in comparison to the blockade produced by atracurium alone (55.7 ± 11.22). These drugs did not alter membrane potential. Lidocaine initially increased the frequency of MEPPs, followed by blockade. With the use of bupivacaine, the blockade was progressive.

☆ Study performed at the Departamento de Anestesiologia da Faculdade de Ciências Médicas da UNICAMP.

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Conclusions: Lidocaine and racemic bupivacaine had a presynaptic effect expressed by alterations in MEPPs, which may explain the interaction and potentiation of NMB produced by atracurium.

PALAVRAS-CHAVE
Anestésicos locais;
Lidocaína;
Bupivacaína racêmica;
Bloqueadores neuromusculares não despolarizantes;
Atracúrio;
Animais;
Ratos

Influência de diferentes anestésicos locais no bloqueio neuromuscular produzido pelo atracúrio em ratos

Resumo

Introdução: A associação de anestésicos locais (AL) com bloqueadores neuromusculares (BNM) na prática clínica e a possibilidade de interação entre esses fármacos têm sido investigadas.
Objetivo: O objetivo do estudo foi avaliar, em modelo experimental, o efeito da lidocaína e da bupivacaína racêmica na transmissão neuromuscular e sua influência no bloqueio neuromuscular produzido pelo atracúrio.
Método: Ratos machos da linhagem Wistar, peso entre 250 e 300 g. A preparação foi realizada de acordo com a técnica proposta por Bulbring. Grupos (n = 5) de acordo com o fármaco em estudo: lidocaína 20 $\mu g.mL^{-1}$ (Grupo I); bupivacaína racêmica 5 $\mu g.mL^{-1}$ (Grupo II); atracúrio 20 $\mu g.mL^{-1}$ (Grupo III); atracúrio 20 $\mu g.mL^{-1}$ em preparação previamente exposta a lidocaína 20 $\mu g.mL^{-1}$ e bupivacaína racêmica 5 $\mu g.mL^{-1}$, Grupos IV e V, respectivamente. Foram avaliadas: 1) A amplitude das respostas do hemidiafragma à estimulação indireta antes e 60 minutos após a adição dos fármacos; 2) Os potenciais de membrana (PM) e os potenciais de placa terminal em miniatura (PPTM).
Resultados: Os AL, isoladamente, não alteraram a amplitude das respostas musculares. Com o uso prévio dos AL, o bloqueio neuromuscular (%) do atracúrio foi 86,66 ± 12,48 e 100, respectivamente, com diferença significante ($p = 0,003$) em relação ao produzido pelo atracúrio isoladamente (55,7 ± 11,22). Não alteraram o PM. A lidocaína inicialmente aumentou a frequência dos PPTM, seguido de bloqueio; com a bupivacaína, o bloqueio foi progressivo.
Conclusões: A lidocaína e a bupivacaína racêmica apresentaram efeito pré-sináptico expresso por alterações nos PPTM, podendo justificar a potencialização do bloqueio neuromuscular produzido pelo atracúrio.

Introduction

The pharmacological properties of neuromuscular blocking (NMB) drugs, such as onset, duration of action and degree of neuromuscular blockade, can be influenced by several factors, such as cardiac output, muscle blood flow, acid-base disorders, temperature, affinity for the site of action, potency and dose administered, as well as drugs used during anesthesia, such as local anesthetics (LA), commonly used in neuraxial techniques in association with general anesthesia.[1–7]

Some authors suggest that high doses of LA *per se* can impair neuromuscular transmission, and therefore potentiate the neuromuscular blockade produced by low doses of NMB drugs.[8]

The mechanism of action of LAs in neuromuscular transmission and their effects on synaptic and electrophysiological components are explained by presynaptic action, inhibiting the release of acetylcholine, and post-synaptic action, through stabilization of the post-junctional membrane, in addition to interference with the muscle fiber excitation-contraction mechanism.[9–12]

Even though only high doses of LAs can change the safety margin of neuromuscular junction transmission, it is of great importance to have better knowledge of their possible interaction with NMBs.[8,13] The objective of the present study was to assess, in an experimental model, the effect of lidocaine and racemic bupivacaine in neuromuscular transmission and its influence on the neuromuscular blockade produced by atracurium.

Method

After approval by the Animal Research Ethics Committee-CEUA – IB – UNICAMP (registered with n° 4672-1/2017 and protocol n° 1204-1), and following the recommendations of CONCEA (National Council for Animal Experimentation Control), male Wistar rats, weighing between 250 and 300 g, were assessed. The animals were anesthetized with isoflurane in a saturated atmosphere and euthanized by deepening anesthesia. Then, they were exsanguinated by section of the neck vessels to facilitate the identification and removal of the left hemidiaphragm and the correspond-

ing phrenic nerve, according to the technique described by Bulbring.[14]

The nerve-muscle preparation was fixed in a vat containing 40 mL of Tyrode nutrient solution and aerated with carbogen (95% O_2 + 5% CO_2) and maintained at 37 °C. The nerve was placed over platinum electrodes connected to a GRASS model stimulator S88. The hemidiaphragm was maintained under constant tension (5 g) by its tendinous portion and subjected to indirect stimulation of 0.1 Hz frequency and 0.2 m.sec^{-1} duration. Tension changes produced by hemidiaphragm contractions were recorded on a Gould RS 3400 physiograph.

For calculating the sample size we used the results of a previous experimental study[15] in which the degree of neuromuscular blockade produced by pancuronium (93.8%) was significantly higher in rats previously exposed to ropivacaine, compared to those unexposed to AL (54.9%), or a difference of 38.9%. Thus, to calculate the sample size, we used the degree of motor blockade as the variable. We arbitrarily considered approximately 85% increase in animals exposed to AL a significant difference in relation to unexposed animals (β error = 20% and α error = 5%), and with a ratio between exposed and unexposed animals of 1:1, we found (n) equal to five rats per group.

The animals were divided into five groups (n = 5), according to the solution added to the preparation: Group Ilidocaine (20 μg.mL^{-1}); Group II – racemic bupivacaine (5 μg.mL^{-1}); Group III – atracurium (20 μg.mL^{-1}); Group IV – atracurium (20 μg.mL^{-1}) in preparation previously exposed to lidocaine (20 μg.mL^{-1}); Group V – atracurium (20 μg.mL^{-1}) in preparation previously exposed to racemic bupivacaine (5 μg.mL^{-1}). The drugs studied were supplied by the Laboratorio Cristália pharmaceutical company.

In the groups in which the combination of drugs was used (Groups IV and V), atracurium was added to the preparation 30 minutes after the addition of LA. Muscle responses to indirect stimulation were recorded for 60 minutes after the addition of NMB.

The hemidiaphragm muscle was used to assess the effects of two local anesthetics, lidocaine (20 μg.mL^{-1}) and racemic bupivacaine (5 μg.mL^{-1}), on membrane potentials (MPs) and miniature end plate potentials (MEPPs). For MPs assessment, the microelectrode was inserted into the superficial fibers of the muscle in sites distant from the terminal motor plate. We performed MP measurements at the following moments: zero (control – before the addition of LA); and at 10, 20, 30, 40, 50 and 60 minutes, after the addition of LA. MEPPs were recorded before (control) and 30 and 60 minutes after addition of LA to the preparation. For MEPPs registration, the microelectrode was inserted as close as possible to the terminal motor plate, and biopotentials were observed with the aid of an oscilloscope and acquired by a microcomputer.

The following data were analyzed: amplitude of muscle responses before and 60 minutes after the addition of lidocaine, racemic bupivacaine and atracurium, alone (Groups I, II and III); amplitude of muscle responses before and 60 minutes after adding atracurium in the preparation previously exposed to the local anesthetics; MPs; and MEPPs. The results were expressed as mean values and standard deviations. For statistical analysis, Kruskal-Wallis tests were used to compare the degree of neuromuscular blockade among the three groups (III, IV and V) and Mann-Whitney tests for comparison between two groups (IV and V). We used the Wilcoxon test to compare muscle fiber MPs registered at the control moment with the remaining moments of the study. A significant level of 5% ($p < 0.05$) was considered.

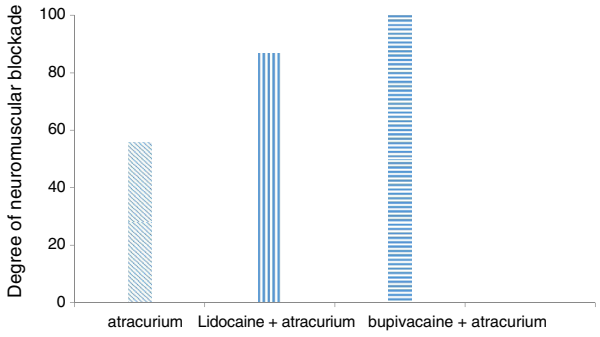

Figure 1 Degree of neuromuscular blockade (%) with atracurium alone and in preparations previously exposed to lidocaine and racemic bupivacaine.

Results

Lidocaine and racemic bupivacaine at the concentrations studied when used alone in rat phrenic nerve-hemidiaphragm preparation did not cause a reduction in the amplitude of muscle responses to indirect electrical stimulation. In the preparations previously exposed to lidocaine and racemic bupivacaine, the neuromuscular blockade produced by atracurium was 86.66% ± 12.48% and 100% ± 0%, respectively, with a significant difference ($p = 0.003$) in relation to the one produced by atracurium used alone (55.7% ± 11.22%) (Fig. 1).

The electrophysiological assessment showed that local anesthetics, at the concentrations used, did not produce changes in the measurements of muscle fiber MPs. Regarding MEPPs, 30 minutes after lidocaine was added to the preparation, an increase in frequency was observed initially, followed by blockade of MEPPs after 60 minutes. Contrary to what was observed with lidocaine, in preparations exposed to racemic bupivacaine, there was a progressive blockade of MEPPs (Fig. 2).

Discussion

Local anesthetics can interfere with neuromuscular function acting on different components of the myoneural junction. Consequently, several mechanisms are involved in the interaction between local anesthetics and neuromuscular blocking drugs. Even though there are many studies trying to explain the direct effects of LA on the neuromuscular plaque, the exact mechanism by which they interfere with neuromuscular transmission remains unclear.

In the presynaptic region, LA can block the conduction of terminal fibers of motor nerves, in addition to inhibiting the release of acetylcholine during nerve stimulation, a fact that has already been observed in several experimental studies investigating different local anesthetics.[9,10,15–19]

Experimental studies using rat phrenic nerve-hemidiaphragm preparation, carried out to assess the

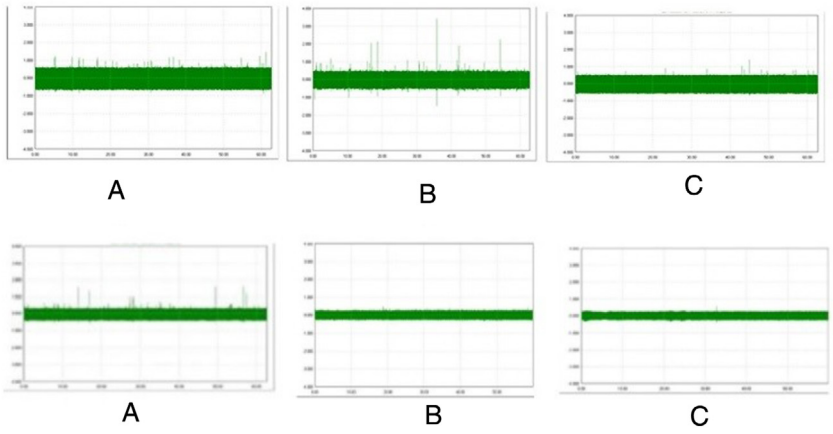

Figure 2 Effects of lidocaine – 20 μg.mL^{-1} (upper line) and racemic bupivacaine – 5 μg.mL^{-1} (lower line), on potentials in miniature endplate potentials in rat diaphragm preparation. A, before addition of local anesthetics (control); B and C, 30 minutes and 60 minutes after addition of local anesthetics.

post-synaptic action of LA, showed results similar to those observed in this study, that is, when used alone, LA did not cause a reduction in the amplitude of muscle response to indirect stimulation. However, in preparations previously exposed to LA, the degree of neuromuscular blockade produced by different NMB drugs was amplified when compared to that resulting from the exclusive use of one of these drugs.[16–18]

In this study, the concentrations of the drugs assessed (lidocaine, bupivacaine and atracurium) were previously tested and determined in previous investigations.[16,20–22]

The present study shows that, in rat phrenic nerve-hemidiaphragm preparation, previously exposed to LA, the blockade produced by atracurium in preparations was significantly greater than the one observed in unexposed preparations.

Clinical studies have also shown the interaction between LA and NMB drugs, resulting in potentiation of neuromuscular blockade produced by NMB drugs.[2–7] In the literature, clinical trials performed by several authors assessed the effects of lidocaine and bupivacaine administered by different routes (epidural and venous), on the features of the neuromuscular blockade produced by atracurium, vecuronium and rocuronium. In patients receiving LA, the authors observed potentiation of neuromuscular blockade shown by longer clinical duration, lower maintenance dose of NMB drug, negative correlation between the plasma concentration of the LA and the ED50 of the NMB drug and longer neuromuscular blockade reversion time after neostigmine use, demonstrating that neuromuscular blockade is more profound in patients who simultaneously receive local anesthetics compared to those submitted only to general anesthesia.[2,3,5,23]

Regarding electrophysiological studies, as previously found in other studies, LA did not have a depolarizing action on the muscle fiber. This feature can be ascertained by examining muscle fiber membrane potentials that remained within normal limits.[16,24]

The assessment of the effect of lidocaine and bupivacaine in the presynaptic region was performed by analyzing the frequency and amplitude of MEPPs in the preparation exposed to LA. There was a reduction in these potentials over time, which demonstrates impairment in the quantal release of the neurotransmitter with decrease in the safety margin of the neuromuscular junction, which may be one of the explanations for the potentiation of NMB drugs. The action of local anesthetics in the postsynaptic region, evaluated in chick biventer cervicis preparations, was demonstrated by a significant decrease in the contracting response to acetylcholine. This may suggest a competitive mechanism between the local anesthetic and the neurotransmitter.[19,24,25]

In addition, LA can bind to several specific acetylcholine sites, desensitize receptors, and temporarily occlude nicotinic receptor channels. The explanation for the interaction between these two classes of drugs, therefore, is multifactorial. LA, by blocking conduction inside the nerve fiber, can interfere with neuromuscular transmission. There is no evidence that they inhibit acetylcholine synthesis, but they reduce the amplitude and frequency of the MEPPs and decrease the release of acetylcholine or decrease the sensitivity of the post-junctional membrane to this neurotransmitter.[9,11,13,14,25,26]

The potentiating effect of the neuromuscular blockade produced by atracurium lidocaine and bupivacaine becomes relevant in clinical practice, since the combination of anesthetic techniques that imply the simultaneous use of local anesthetics and neuromuscular blocking drugs can cause undesirable effects during and after surgical procedures.

Study limitation

As it is an experimental study in rats, and despite the absence of bias in the experiments carried out, we cannot quantitatively extrapolate to clinical evidence. In other words, although this interaction may differ among species and the extrapolation of our results to humans is not quantitatively similar, the clinical implications of the interaction make it essential to monitor neuromuscular function, which is the most appropriate method to assess the correct and safe use of these drugs.

Conflicts of interest

The authors declare no have conflicts of interest.

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References

1. Donati F. Onset of action of relaxants. Can J Anaesth. 1988;35:52–8.
2. Toft P, Kirkegaard Nielsen H, Severinsen I, et al. Effect of epidurally administered bupivacaine on atracurium-induced neuromuscular blockade. Acta Anaesthesiol Scand. 1990;34:649–52.
3. Taivainen T, Meretoja OA, Rosenberg PH. The effect of epidural bupivacaine on vecuronium-induced neuromuscular blockade in children. Acta Anaesthesiol Scand. 1994;38:453–6.
4. Nonaka A, Sugawara T, Suzuki S, et al. Pretreatment with lidocaine accelerates onset of vecuronium-induced neuromuscular blockade. Masui. 2002;51:880–3.
5. Munakata K, Suzuki T, Watanabe N, et al. Influence of epidural lidocaine injection on vecuronium-induced neuromuscular blockade. Masui. 2004;53:1377–80.
6. Suzuki T, Mizutani H, Ishikawa K, et al. Epidurally administered mepivacaine delays recovery of train-of-four ratio from vecuronium-induced neuromuscular block. Br J Anaesth. 2007;99:721–5.
7. Sahin SH, Colak A, Sezer A, et al. Effect of epidural levobupivacaine on recovery from vecuronium-induced neuromuscular block in patients undergoing lower abdominal surgery. Anaesth Intensive Care. 2011;39:607–10.
8. Telivuo L, Katz RL. The effects of modern intravenous local analgesics on respiration during partial neuromuscular block in man. Anaesthesia. 1970;25:30–5.
9. Usubiaga JE, Wikinski JA, Morales RL. Interaction of intravenously administered procaine, lidocaine and succinylcholine in anesthetized subjects. Anesth Analg. 1967;46:39–45.
10. Matsuo S, Rao DB, Chaudry I, et al. Interaction of muscle relaxants and local anesthetics at the neuromuscular junction. Anesth Analg. 1978;57:580–7.
11. Neher E, Steinbach JH. Local anaesthetics transiently block currents through single acetylcholine-receptor channels. J Physiol. 1978;277:153–76.
12. Sine SM, Taylor P. Local anesthetics and histrionicotoxin are allosteric inhibitors of the acetylcholine receptor. Studies of clonal muscle cells. J Biol Chem. 1982;257:8106–14.
13. Pederneiras SG. Interação de drogas com relaxants musculares. Rev Bras Anestesiol. 1988;38:63–73.
14. Bulbring E. Observation on the isolated phyrenic nerve-diaphragm preparation of the rat. Br J Pharmacol. 1946;1:38–61.
15. Braga Ade F, Carvalho VH, Braga FS, et al. Effect of ropivacaine combined with pancuronium on neuromuscular transmission and effectiveness of neostigmine and 4-aminopyridine for blockade reversal: experimental study. Rev Bras Anestesiol. 2015;65:136–40.
16. Carvalho VH, Braga Ade F, Braga FS, et al. The influence of lidocaine and racemic bupivacaine on neuromuscular blockade produced by rocuronium. A study in rat phrenic nerve-diaphragm preparation. Acta Cir Bras. 2009;24:211–5.
17. Carvalho VH, Braga Ade F, Braga FS, et al. Association between levobupivacaine and pancuronium. Interference in neuromuscular transmission and blockade in rats. Acta Cir Bras. 2016;31:486–9.
18. Braga Ade F, Carvalho VH, da Silva Braga FS, et al. Effect of 50% enantiomeric excess bupivacaine mixture combined with pancuronium on neuromuscular transmission in rat phrenic nerve-diaphragm preparation; a pilot study. Indian J Anaesth. 2015;59:701–5.
19. Braga Ade F, Carvalho VH, Braga FS, et al. Evidence of presynaptic and postsynaptic action of local anesthetics in rats. Acta Cir Bras. 2013;28:774–7.
20. De Barcelos CC, Braga Ade F, Braga FS, et al. In vitro and in vivo neuromuscular effects of atracurium and rocuronium in rats treated with carbamazepine for seven days. Rev Bras Anestesiol. 2008;58:137–51.
21. Fernandes SC, Braga Ade F, Braga FS, et al. Influence of lithium on the neuromuscular blockade produced by atracurium and cisatracurium: study on rat phrenic nerve-diaphragm preparations. Rev Bras Anestesiol. 2007;57:289–300.
22. Sousa SR, Braga Ade F, Potério GM, et al. Influence of nifedipine on the neuromuscular block produced by atracurium and cistracurium: study in rat phrenic-diaphragmatic nerve preparation. Rev Bras Anestesiol. 2006;56:157–67.
23. Cardoso LS, Martins CR, Tardelli MA. Effects of intravenous lidocaine on the pharmacodynamics of rocuronium. Rev Bras Anestesiol. 2005;55:371–80.
24. Bowman WC. Prejunctional and postjunctional cholinoceptors at the neuromuscular junction. Anesth Analg. 1980;59:935–43.
25. Braga Ade F, Carvalho VH, Braga FS, et al. Influence of local anesthetics on the neuromuscular blockade produced by rocuronium: effects of lidocaine and 50% enantiomeric excess bupivacaine on the neuromuscular junction. Rev Bras Anestesiol. 2009;59:725–34.
26. Usubiaga JE, Standaert F. The effect of local anesthetics on motor nerve terminals. J Pharmacol Exp Ther. 1968;159:353–61.