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SCIENTIFIC ARTICLE

Effect of ropivacaine combined with pancuronium on neuromuscular transmission and effectiveness of neostigmine and 4-aminopyridine for blockade reversal: experimental study☆

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Local anesthetics, ropivacaine;
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Nondepolarising, pancuronium;
Animals, rats

Abstract
Background and objectives: The local anesthetic effects on neuromuscular junction and its influence on blockade produced by nondepolarizing neuromuscular blockers are still under-investigated; however, this interaction has been described in experimental studies and in humans. The aim of this study was to evaluate in vitro the interaction between ropivacaine and pancuronium, the influence on transmission and neuromuscular blockade, and the effectiveness of neostigmine and 4-aminopyridine to reverse the blockade.
Methods: Rats were divided into groups ($n = 5$) according to the study drug: ropivacaine ($5\,\mu g\,mL^{-1}$); pancuronium ($2\,\mu g\,mL^{-1}$); ropivacaine + pancuronium. Neostigmine and 4-aminopyridine were used at concentrations of $2\,\mu g\,mL^{-1}$ and $20\,\mu g\,mL^{-1}$, respectively. The effects of ropivacaine on membrane potential and miniature endplate potential, the amplitude of diaphragm responses before and 60 min after the addition of ropivacaine (degree of neuromuscular blockade with pancuronium and with the association of pancuronium–ropivacaine), and the effectiveness of neostigmine and 4-aminopyridine on neuromuscular block reversal were evaluated.

☆ Study performed at the Department of Anesthesiology and Pharmacology, Campinas State University (UNICAMP) School of Medicine, Campinas, SP, Brazil.

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Results: Ropivacaine did not alter the amplitude of muscle response (the membrane potential), but decreased the frequency and amplitude of the miniature endplate potential. Pancuronium blockade was potentiated by ropivacaine, and partially and fully reversed by neostigmine and 4-aminopyridine, respectively.
Conclusions: Ropivacaine increased the neuromuscular block produced by pancuronium. The complete antagonism with 4-aminopyridine suggests presynaptic action of ropivacaine.

PALAVRAS-CHAVE
Anestésicos Locais, ropivacaina;
Bloqueadores neuromusculares;
Não despolarizantes, pancurônio;
Animais, ratos

Efeito da associação ropivacaina-pancurônio na transmissão neuromuscular e eficácia da neostigmine e 4-aminopiridina na reversão do bloqueio: estudo experimental

Resumo
Justificativa e objetivos: Os efeitos dos anestésicos locais na junção neuromuscular e sua influência no bloqueio produzido por bloqueadores neuromusculares não-despolarizantes é ainda alvo de pouca investigação, no entanto esta interação tem sido descrita em trabalhos experimentais e em humanos. O objetivo deste estudo foi avaliar in vitro, a interação da ropivacaína com o pancurônio, a influência na transmissão e bloqueio neuromuscular e a efetividade da neostigmina e 4-aminopiridina na reversão do bloqueio.
Método: Ratos foram distribuídos em grupos (n = 5) de acordo com o fármaco estudado: ropivacaína (5 $\mu g\,mL^{-1}$); pancurônio (2 $\mu g\,mL^{-1}$); ropivacaína + pancurônio. A neostigmina e a 4-aminopiridina foram usadas nas concentrações de 2 $\mu g\,mL^{-1}$ e 20 $\mu g\,mL^{-1}$, respectivamente. Avaliou-se: 1) efeitos da ropivacaína sobre o potencial de membrana e potenciais de placa terminal em miniatura; 2) a amplitude das respostas do diafragma antes e 60 minutos após a adição da ropivacaína; o grau de bloqueio neuromuscular com o pancurônio e com a associação pancurônio - ropivacaína; 3) a efetividade da neostigmina e 4-aminopiridina na reversão do bloqueio neuromuscular.
Resultados: A ropivacaína não alterou a amplitude das respostas musculares, os potenciais de membrana, mas diminuiu a frequência e a amplitude dos potenciais de placa terminal em miniatura. O bloqueio produzido pelo pancurônio foi potencializado pela ropivacaína, e parcial e totalmente revertido pela neostigmina e 4-aminopiridina, respectivamente.
Conclusões: A ropivacaína potencializou o bloqueio neuromuscular produzido pelo pancurônio. O antagonismo completo com a 4-aminopiridina sugere ação pré-sináptica da ropivacaína.

Introduction

Local anesthetics, particularly amino amides, are a group of drugs widely administered by different routes, such as topical, subcutaneous infiltration, peripheral nerve block, neuraxial anesthesia alone or combined with general anesthesia.[1–4]

There is evidence that these drugs may interfere with neuromuscular transmission and increase the effects of neuromuscular blockers.[1–7]

Ropivacaine is an amino-amide local anesthetic with similar physicochemical properties to bupivacaine (S50%–R50%), except for the lower potency and lesser degree of motor blockade, with greater selectivity for sensory nerve fibers, characteristics attributed to its lower lipid solubility and pure S^- isomer structure as opposed to the racemic mixture of bupivacaine.[8,9]

These characteristics are also responsible for less cardiac and central nervous system toxicity, ropivacaine advantages over bupivacaine (S50%–R50%).[8,9] Pancuronium is a long acting nondepolarizing aminostreroid neuromuscular blocker, which justifies its use in prolonged surgery and intensive care.[10]

The aim of this study was to evaluate in an experimental model the effect of ropivacaine on neuromuscular transmission, its influence on the neuromuscular block produced by pancuronium, and the effectiveness of neostigmine and 4-aminopyridine on blockade reversal.

Method

This is an in vitro experimental study in which the procedures used were in accordance with the ethical principles

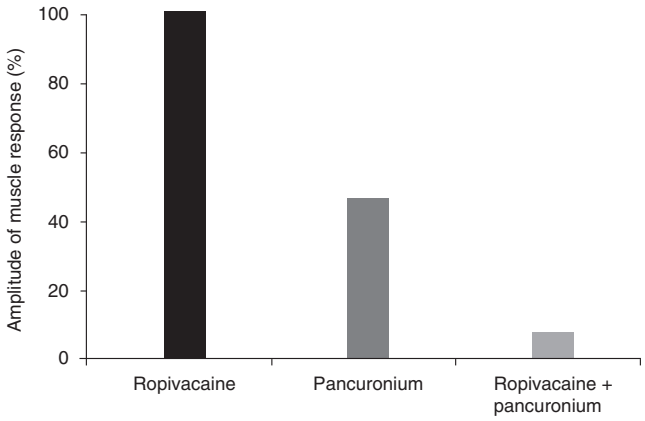

Figure 1 Amplitude of muscle response to indirect stimulation on phrenic nerve-diaphragm preparation of rats exposed to ropivacaine (5.0 $\mu g\,mL^{-1}$), pancuronium (2 $\mu g\,mL^{-1}$), and ropivacaine + pancuronium.

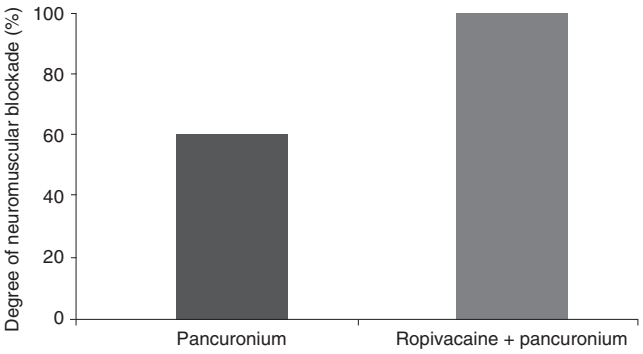

Figure 2 Degree of blockade with pancuronium alone and in preparation previously exposed to ropivacaine.

of animal experimentation adopted by the Brazilian College of Animal Experimentation (COBEA), approved by the Animal Research Ethics Committee of the Institute of Biology, Campinas State University (protocol No. 2346-1).

Male Wistar rats weighing between 180 and 250 g were used. The animals were anesthetized intraperitoneally with urethane (1.2 $mg\,kg^{-1}$), followed by exsanguination by a section of the neck vessels to facilitate identification and removal of the left hemidiaphragm and the phrenic nerve corresponding portion. Bulbring[11] technique was used to evaluate the effect of ropivacaine on neuromuscular transmission, its influence on blockade produced by pancuronium and the effectiveness of neostigmine and 4-aminopyridine on neuromuscular blockade reversal. The preparations were fixed in a vat containing 40 mL nutritious Tyrode solution, continuously 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 S48 stimulator. The diaphragm was maintained, by its tendinous portion, under constant voltage (5.0 g) via wire connected to isometric transducer Load Cell BG50 GMS and subjected to indirect stimulation of 0.1 Hz frequency and duration of 0.2 ms, and the voltage variations produced by diaphragm contractions were recorded in physiograph Gould RS 3400. To evaluate the effect of drugs used alone and in combination on neuromuscular transmission, three groups were formed ($n = 5$): Group I, ropivacaine (5 $\mu g\,mL^{-1}$); Group II, pancuronium (2 $\mu g\,mL^{-1}$); and Group III, pancuronium (2 $\mu g\,mL^{-1}$) in preparation previously exposed to ropivacaine (5 $\mu g\,mL^{-1}$). In Group III (pancuronium–ropivacaine), pancuronium was added to the preparation 30 min after the addition of ropivacaine. Muscle response to indirect stimulation was recorded for 60 min after addition of the drugs.

The same preparation was used to study the effectiveness of the drugs (neostigmine – 2 $\mu g\,mL^{-1}$ and 4-aminopriridina – 20 $\mu g\,mL^{-1}$) on neuromuscular blockade reversal, which were added to the preparation after the blockade produced by ropivacaine-pancuronium combination. In the rat diaphragm, the effects of ropivacaine on miniature endplate potentials and membrane potentials were also studied. Parameters evaluated were (1) extent of diaphragm muscle response to indirect stimulation before and 60 min after ropivacaine addition; (2) extent of diaphragm muscle response to indirect stimulation before and 60 min after pancuronium addition, alone and previously combined with ropivacaine; (3) membrane potentials (MP) and miniature endplate potentials (MEPP); and (4) effectiveness of neostigmine and 4-aminopyridine on neuromuscular blockade reversal.

Results were expressed as means and standard deviations. Wilcoxon test was used to analyze the membrane potential of muscle fiber and the effectiveness of neuromuscular blockade reversal drugs. To evaluate the reduction in the extent of muscle response, Student's *t*-test (normal distribution) was used. A significant level of 5% ($p < 0.05$) was assumed. The power of the test was calculated and $\beta > 20\%$ (power > 80%) was obtained.

Results

At the concentration studied and used alone, ropivacaine did not reduce the extent of muscle response to indirect electrical stimulation on rat phrenic nerve-diaphragm. With pancuronium alone and in preparations previously exposed to ropivacaine, the mean extent of muscle responses was 45.1% and 6.2%, respectively, and the corresponding blockade was 54.9 ± 14.1 and $93.8\% \pm 9.2\%$, respectively, with significant difference ($p = 0.015$) (Figs. 1 and 2).

The neuromuscular blockade caused by pancuronium in preparations exposed to ropivacaine was both partially and fully reversed by neostigmine and 4-aminopyridine, respectively.

There was no significant effect of ropivacaine on membrane potentials (Fig. 3). Effects on miniature endplate potentials (MEPP) were characterized by a decrease in frequency and extent until complete blockade.

Discussion

The effects of local anesthetics on neuromuscular junction and its influence on the blockade produced by nondepolarizing neuromuscular blockers are still under-investigated; however, this interaction has been described in experimental and human studies.[1–7,12] Experimental studies[5–7,13] serve as the basis for the results observed in the clinic, with the

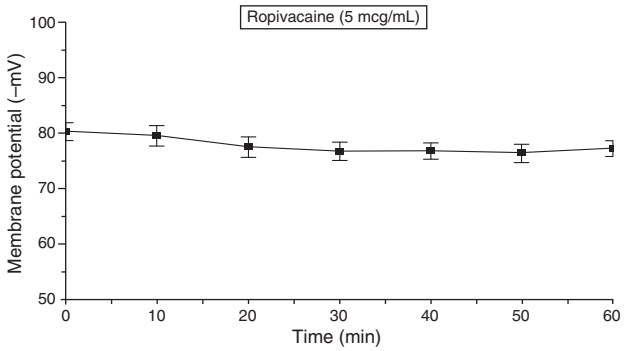

Figure 3 Ropivacaine (5.0 μg mL^{-1}) effect on membrane potential in the rat diaphragm preparation.

advantage of eliminating bias, which is the great individual variability in response to neuromuscular blockers.[10,14]

Although local anesthetics can only produce neuromuscular blockade at high doses, interactions with neuromuscular blockers, particularly non-depolarizing, become clinically relevant, and careful observation is required when using these agents simultaneously, or in situations where the safety margin of neuromuscular transmission is reduced.[1–4,12,14,15]

Several mechanisms are admitted to explain the interaction between local anesthetics and neuromuscular blockers: in the presynaptic region, it selectively depresses conduction in motor fibers and inhibits the acetylcholine release during nerve stimulation; at the postsynaptic level, local anesthetics may bind to different specific acetylcholine sites, resulting in desensitization of receptors, and may cause temporary occlusion of nicotinic receptor channels; furthermore, a stabilizing action of postjunctional membrane and the interference with the muscular fiber excitation-contraction mechanism are described.[5–7,11,13,16–19]

The ropivacaine concentration used was established in a pilot study and determined from data presented in studies carried out in Brazil, where other amino-amide local anesthetics with similar characteristics to ropivacaine were used.[6,7] Matsuo et al.[13] evaluated, in preparation similar to that used in this study, the association of d-tubocurarine with different local anesthetics and found that, even in ineffective concentrations, local anesthetics potentiated neuromuscular blocker, as evidenced by significant decrease in the ED50. Regarding the influence of neuromuscular blockers on the effects local anesthetics, these authors also reported that ineffective concentrations of d-tubocurarine caused a similar decrease of ED50 and increase of local anesthetic potency.

In a clinical trial, Sahin et al.[4] evaluated the characteristics of neuromuscular block produced by vecuronium in patients undergoing general anesthesia combined with epidural block with 0.5% levobupivacaine (15 mL) and observed a significant increase in the rate of recovery and total duration of vecuronium effect, without, however, influencing its clinical duration (CD25%). These findings may be explained by the fact that levobupivacaine metabolism, when used in epidural space, only occurs in approximately 30 min when the drug reaches the circulation.[1]

The present study showed that ropivacaine, at the concentration studied, administered alone had no effect on neuromuscular junction; however, it potentiated the blockade produced by pancuronium. These results are similar to those of other authors, who found no clinical impairment in neuromuscular transmission in experimental studies with the isolated use of different local anesthetics. However, a clear potentiation of the effect of various neuromuscular blockers has been described as a result of these drugs combination, an interaction that may be consequential to the true potentiation at different locations of the neuromuscular junction,[1,2,4–7,12,13,20] caused by the action of the two drugs

It is believed that the greatest degree of neuromuscular blockade caused by pancuronium in rat diaphragm preparations previously exposed to ropivacaine, and evidenced by a greater reduction in the extent of muscle responses to phrenic nerve stimulation, is due to a presynaptic action of ropivacaine and not to the muscular fiber depolarizing action, as it was found in electrophysiological studies that bupivacaine at the concentration used did not modify the membrane potential of muscle fibers. The presynaptic action was demonstrated by the decrease in the frequency and amplitude of miniature endplate potentials (MEPP) caused by ropivacaine, being the result of changes in quantal release of acetylcholine.

The neuromuscular blockade caused by ropivacaine combined with pancuronium was completely reversed by 4-aminopyridine and, to a lesser extent, with neostigmine. These results were also described by Sahin et al.[4] who observed greater efficacy of 4-aminopyridine in humans compared to neostigmine on blockade reversal caused by vecuronium in patients receiving levobupivacaine in the epidural space. In experimental studies, similar results were found regarding reversal of blockade caused by lidocaine–rocuronium combination.[5]

By inhibiting the acetylcholinesterase, neostigmine increases the neurotransmitter concentration in the synaptic cleft, competitively displacing the agents causing blockage. The partial antagonism of neostigmine reinforces this finding, as cholinesterase inhibitors are only effective in reversing the postsynaptic block. The 4-aminopyridine, in addition to its inhibitory effect of endplate nicotinic receptor desensitization, causes increased quantal acetylcholine. This increase is the result of actions in the membrane of nerve endings, such as potassium channel inhibition, which produces an increase in the duration of the action potential and increased influx of calcium ions to motor nerve endings during membrane depolarization.[21–23] The complete antagonism achieved with 4-aminopyridine suggested that ropivacaine interaction with pancuronium has presynaptic component related to decreased acetylcholine release.

Ropivacaine alone did not compromise neuromuscular transmission, but potentiated the blockade produced by pancuronium, which was reversed by neostigmine and 4-aminopyridine. These findings are important for clinical practice because it provides guidance on the need for monitoring, particularly when combined with other drugs.

Conflicts of interest

The authors declare no conflicts of interest.

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