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

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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-aminopyrididine to reverse the blockade.

Methods: Rats were divided into groups (n=5) according to the study drug: ropivacaine (5 μg mL⁻¹); pancuronium (2 μg mL⁻¹); ropivacaine + pancuronium. Neostigmine and 4-aminopyrididine were used at concentrations of 2 μg mL⁻¹ and 20 μg 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-aminopyrididine on neuromuscular block reversal were evaluated.
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 (550%-850%), 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

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.

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

Efeito da associação ropivacaina-pancurônio na transmissão neuromuscular e eficácia da neostigmina 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 ropivacaina 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: ropivacaina (5 µg mL⁻¹); pancurônio (2 µg mL⁻¹); ropivacaina + pancurônio. A neostigmina e a 4-aminopiridina foram usadas nas concentrações de 2 µg mL⁻¹ e 20 µg mL⁻¹, respectivamente. Avaliou-se: 1) efeitos da ropivacaina sobre o potencial de membrana e potenciais de placa terminal em miniatura; 2) a amplitude das respostas do diafragma antes e após 60 minutos após a adição da ropivacaina; o grau de bloqueio neuromuscular com o pancurônio e com a associação pancurônio - ropivacaina; 3) a efetividade da neostigmina e 4-aminopiridina na reversão do bloqueio neuromuscular.

Resultados: A ropivacaina 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 ropivacaina, e parcial e totalmente revertido pela neostigmina e 4-aminopiridina, respectivamente.

Conclusões: A ropivacaina potencializou o bloqueio neuromuscular produzido pelo pancurônio. O antagonismo completo com a 4-aminopiridina sugere ação pré-sináptica da ropivacaina.

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These characteristics are also responsible for less cardiac and central nervous system toxicity, ropivacaine advantages over bupivacaine (550%-850%).8,9 Pancuronium is a long acting nondepolarizing aminoesteroid 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
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\(^{1}\) 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 nutritive Tyrode solution, continuously aerated with carbogen (95% \(\text{O}_2\) + 5% \(\text{CO}_2\)) 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 V) 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-aminopyridine – 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

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 were 54.9 ± 14.1 and 93.8% ± 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 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.
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,1,2,4-7,12,13,19 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 blockade. 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.
References


