

RESEARCH ARTICLE

Reversal of neuromuscular block produced by local anaesthetics combined with pancuronium. Experimental study.

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Abstract

Justification and objectives: There are few clinical and experimental studies on the interaction between local anaesthetics and nondepolarising neuromuscular blocking agents and their effects on the neuromuscular junction. However, potentiation of these drugs has been observed when they are used simultaneously. The aim of this study was to evaluate in vitro the efficacy of neostigmine and 4-aminopyridine in the reversal of a neuromuscular block produced by pancuronium combined with different local anaesthetics.

Method: Rats were distributed in groups (n = 5), according to drug combinations: pancuronium + levobupivacaine and pancuronium + enantiomeric mixture. The concentrations of levobupivacaine, enantiomeric mixture and pancuronium were respectively 5µg.mL⁻¹, 5µg.mL⁻¹ and 2µg.mL⁻¹. Neostigmine and 4-aminopyridine were used in concentrations of 2µg.mL⁻¹ and 20µg.mL⁻¹, respectively. The following parameters were evaluated: 1) degree of neuromuscular block obtained by a combination of pancuronium + levobupivacaine and pancuronium + enantiomeric mixture; 2) efficacy of neostigmine and 4-aminopyridine in the reversal of neuromuscular block produced by drug combinations.

Results and Conclusion: Neostigmine and 4-aminopyridine were effective at reversing neuromuscular block obtained by different drug combinations, and the block was partially and totally reversed with neostigmine and 4-aminopyridine, respectively.

Keywords: Local anaesthetics, neuromuscular junction, neuromuscular blocking drugs

Introduction

The mechanism of action of local anaesthetics, their effects on synaptic and electrophysiological components of the nerve cell, influence on the pharmacodynamics of different neuromuscular blockers and reversal of neuromuscular block observed when these drugs are combined with local anaesthetics, have still not been fully investigated.¹⁻⁴ In clinical practice, respiratory complications due to deep and residual neuromuscular block are frequent and feared events. Anaesthetic recovery may be prolonged, increasing the demand for more beds in Intensive Therapy Units, a longer hospital stay, increased costs and higher patient morbidity and mortality.^{5,6}

Levobupivacaine is a 100% (S-) pure enantiomer of racemic bupivacaine (S50-R50). As a result, it has a lower lipid solubility and lower cardio/neurotoxicity.⁷ The enantiomeric mixture resulted from manipulation of racemic bupivacaine components containing 25% of bupivacaine R isomer R (+) and 75% of S isomer (-).^{8,9} Pancuronium is a long-acting non-depolarising aminosteroid neuromuscular blocking agent. This fact justifies its use in prolonged surgeries and intensive care.^{10,11} The aim of this study was to assess the efficacy of neostigmine and 4-aminopyridine in the reversal of neuromuscular

block produced by pancuronium combined with levobupivacaine and enantiomeric mixture, using an experimental model.

Method

This is an in vitro experimental study, conducted in compliance with ethical guidelines on animal experimentation, followed by the Brazilian College of Animal Experimentation (COBEA) and approved by the Ethics Committee in Animal Experimentation of the Institute of Biology of the State University of Campinas (protocol number 2346-1). Wistar male rats (weight: 180-250 g) were used. The animals were anaesthetized with urethane (1.2 mg.kg⁻¹, by intraperitoneal route), then exsanguinated by sectioning of neck vessels, to facilitate the identification and removal of the left hemidiaphragm and a portion of the correspondent phrenic nerve. The technique described by Bulbring¹² was used to assess the influence of different local anaesthetics (levobupivacaine and enantiomeric mixture) on the neuromuscular block produced by pancuronium and the efficacy of neostigmine and 4-aminopyridine in the reversal of neuromuscular block obtained with a combination of drugs. Preparations were fixed in a vat containing 40 mL of Tyler nutrient solution, constantly aerated with carbogen (95% O₂ + 5% CO₂) and maintained at 37°C. The nerve was placed over

platinum electrodes connected to a Grass S48 stimulator. The tendinous portion of the diaphragm was maintained under constant tension (5.0 g), through a wire connected to a Load Cell BG50 GMS isometric transducer. Indirect stimulation was applied at a frequency of 0.1 Hz and duration of 0.2 m.sec. Variations in tension produced by diaphragm contractions were recorded in a Gould RS 3400 physiographer. To determine the neuromuscular block produced by a combination of drugs, the following groups were formed (n = 5): Group I - pancuronium ($2\mu\text{g.mL}^{-1}$) + levobupivacaine ($5\mu\text{g.mL}^{-1}$); Group II - pancuronium ($2\mu\text{g.mL}^{-1}$) + enantiomeric mixture ($5\mu\text{g.mL}^{-1}$). Neostigmine ($2\mu\text{g.mL}^{-1}$) and 4-aminopyridine ($20\mu\text{g.mL}^{-1}$) were used for the reversal of neuromuscular block in different combinations with new preparations (n=5). Pancuronium was added 30 minutes after exposure of the preparation to local anaesthetics. The degree of neuromuscular block produced was assessed at 60 minutes of drug exposure, at the time when neostigmine or 4-aminopyridine were added to the preparation, and drug efficacy was evaluated in the reversal of neuromuscular block. The parameters evaluated were: 1) the amplitude of response of the diaphragm muscle to indirect stimulation before and 60 minutes after the addition of pancuronium in a preparation previously exposed to levobupivacaine and enantiomeric mixture; 2) the effectiveness of neostigmine and 4-aminopyridine in the reversal of neuromuscular block obtained by drug combinations. The results were expressed as means and standard deviations. Wilcoxon's test was used for analysis of drug efficacy in the reversal of neu-

romuscular block. To assess the degree of block with different combinations (pancuronium + local anaesthetics), Student's t-test was used (normal distribution). A significance level of 5% was adopted ($p < 0.05$). The power of the test was calculated and achieved $> 20\%$ (power $> 80\%$).

Results

In a rat phrenic nerve-diaphragm preparation, muscle response to indirect electrical stimulation did not display any reduction in amplitude, when exposed during 30 minutes to local anaesthetics only (levobupivacaine and enantiomeric mixture). Values of the mean and standard deviation of the degree of blockade produced by pancuronium in preparations previously exposed to levobupivacaine and enantiomeric mixture, were respectively: $90.2 \pm 18.4\%$ and 100% , without any significant difference ($p=0.44$). Values of mean and standard deviation of the amplitude of muscle response after the addition of neostigmine and 4-aminopyridine, for the reversal of neuromuscular block produced by the combination of pancuronium + levobupivacaine, were respectively, $75.6 \pm 10.2\%$ and 100% , with a significant difference ($p = 0.0003$). For the reversal of neuromuscular blockade in preparations exposed to pancuronium + S75-R25, the amplitudes of response after neostigmine and 4-aminopyridine, were $83.3 \pm 6.8\%$ and 100% , respectively, with a significant difference ($p= 0.0001$). Neuromuscular block caused by pancuronium in preparations exposed to levobupivacaine and enantiomeric mixture was partially and totally

reversed by neostigmine and 4-aminopyridine, respectively.

Discussion

In experimental studies previously performed in our setting, it was observed that the block produced by pancuronium alone was approximately 54.9%, and was potentiated by ropivacaine, resulting in a neuromuscular block of 93.8% in preparations previously exposed to this local anaesthetic.⁴ In this study, levobupivacaine and enantiomeric mixture in concentrations employed and previously added to a phrenic nerve-diaphragm preparation, potentiated the block produced by pancuronium.

This interaction and potentiation of the pharmacodynamic effects of neuromuscular blocking agents have also been shown in clinical^{13,14} and experimental trials.^{4,7,15} In human studies, local anaesthetics were employed by the epidural route, and a longer recovery time for neuromuscular transmission was observed both spontaneously and after use of neostigmine for the reversal of neuromuscular block. Sahin *et al.*,¹³ studied the effects of levobupivacaine by the epidural route on the neuromuscular block produced by vecuronium and observed that spontaneous recovery from the block was significantly higher in patients receiving epidural block associated with general anaesthesia, in comparison to patients who were not exposed to local anaesthetics. Similar results have been previously described by other authors.¹⁴ Those authors observed that time for the reversal of neuromuscular block produced by atracurium after neostigmine use was significantly longer in patients receiving bupiva-

caine simultaneously by the epidural route, than in patients undergoing only general anaesthesia. It can be inferred from these results that local anaesthetics not only potentiate neuromuscular block, but also make both spontaneous and pharmacological reversal more difficult.

In this study, a neuromuscular block produced by local anaesthetics combined with pancuronium was reversed by neostigmine and 4-aminopyridine. However, neostigmine was less effective at reversing neuromuscular block than 4-aminopyridine. With the latter drug, the amplitude of muscle response to phrenic nerve stimulation exceeded the response considered as control. The complete reversal observed with 4-aminopyridine may suggest that the interaction between these local anaesthetics and pancuronium is attributed to a presynaptic action of local anaesthetics, as already shown in a previous study. In that study, a decrease in the amplitude and frequency of miniature end-plate potentials was observed in preparations exposed to these drugs.²

Neostigmine is among the most widely used anticholinesterases in clinical practice. It inhibits acetylcholinesterase, increases the concentration of neurotransmitter at the synaptic cleft and can competitively dissociate agents causing the neuromuscular block. Although it is not employed in clinical practice, 4-aminopyridine may reverse the effects of neuromuscular blocking agents. This activity may be explained by different mechanisms at nerve ending membranes such as: inhibition of potassium channels, producing increased duration of the action potential and

a higher influx of calcium ions to motor nerve endings during membrane depolarisation. Consequently, desensitisation of nicotinic receptors is inhibited at the terminal plate, provoking a considerable increase in acetylcholine quanta. 4-AP may directly activate voltage-dependent presynaptic calcium channels, potentiating neurotransmitter release, irrespective of K channel block. Its use in clinical practice is not recommended, since it readily crosses the blood-brain barrier with stimulation of the central nervous system, and may cause seizures.¹⁶⁻²⁰

Results have demonstrated the efficacy of reversal agents in the antagonism of neuromuscular block produced by local anaesthetics combined with pancuronium. Therefore, we can understand the importance of this interaction in clinical practice, resulting in potentiation of a neuromuscular block. The reversal and monitoring of neuromuscular block is vital for greater patient safety.

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