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Paradoxical neostigmine-induced TOF_{fade}: On the role of presynaptic cholinergic and adenosine receptors

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ABSTRACT

Neuromuscular transmission is clinically monitored using the train-of-four ratio (TOF_{ratio}), which is the quotient between twitch tension produced by the fourth (T_4) and by the first (T_1) stimulus within a train-of-four stimulation at 2 Hz. Neostigmine causes a paradoxical depression of the TOF_{ratio} (TOF_{fade}) that is amplified by intra-arterial atropine in cats. This led us to question the usefulness of the TOF_{ratio} as a sole testing element to control neostigmine-induced reversal of neuromuscular transmission block caused by non-depolarizing agents. We hypothesized that the inhibition of cholinesterase activity might increase acetylcholine bioavailability and consequently cholinoceptor activation, leading to concomitant adenosine release from nerve endings and skeletal muscle fibers. The involvement of presynaptic muscarinic and adenosine receptors in neostigmine-induced TOF_{fade} in the rat phrenic nerve diaphragm was investigated. Blockade of adenosine A_{2A} receptors with ZM241385 and of muscarinic M_2 receptors with methoctramine or atropine amplified neostigmine-induced TOF_{fade}. Notwithstanding TOF_{fade} amplification, the blockade of M_2 or A_{2A} receptors increased both T_1 and T_4 twitch tensions above control during the first 3 min of neostigmine application. Beyond that period, the T_1 twitch tension returned to baseline, whereas T_4 decreased further until the control value with neostigmine alone. Blockade of M_1 receptors by pirenzepine did not change neostigmine-induced TOF_{fade}, unless A_{2A} receptors were concurrently blocked with ZM241385. Data indicate that the paradoxical neostigmine-induced fade involves presynaptic mechanisms that regulate transmitter release and synaptic adenosine accumulation, including the activation of adenosine A_{2A} and muscarinic M_2 receptors.

1. Introduction

The magnitude of neuromuscular transmission blockade is clinically monitored using the train-of-four ratio (TOF_{ratio}). The TOF_{ratio} is the quotient between muscular tension produced by the fourth stimulus (T_4) and the first stimulus (T_1) within a train-of-four stimulus delivered at 2 Hz. Reductions of the TOF_{ratio} are referred to as TOF_{fade} and indicate the degree of patients' curarization (Ali et al., 1970; Murphy, 2006). Antinicotinic agents cause TOF_{fade} by decreasing the quantal output (Matzner et al., 1988) without changing endplate resting potentials (Magleby et al., 1981). Twitch blockade and neurotransmission fade caused by antinicotinic agents are determined by separate, independent actions of drugs. However, an increase in the frequency of nerve stimulation or the use of neostigmine may significantly change this scenario. An increase in cholinergic neurotransmission/neuromodulation that differentially affects T_1 and T_4 twitch tensions might occur under such conditions. In fact, a paradoxical reduction of the TOF_{ratio} caused by neostigmine has been recorded *in vivo* in the cat sciatic nerve tibial muscle preparation, and neostigmine-induced decrease in TOF_{ratio} was augmented by atropine *via* a yet undisclosed mechanism (Alves-do-Prado et al., 1989). Likewise, we found differences in the magnitude of tetanic fade and TOF_{fade} produced by antinicotinic muscular relaxants, depending on whether the compounds also exhibited anticholinesterase activity (Bornia et al., 2009, 2011; Pereira et al., 2011).

Acetylcholine activates nicotinic receptors that contain α_1-subunits in skeletal muscle fibers, causing muscular contractions, but this neurotransmitter may also facilitate its own release by activating fast-desensitizing facilitatory nicotinic autoreceptors (Colquhoun et al., 1989; Wessler et al., 1986) that express $\alpha 3\beta 2$ subunits (Faria et al., 2003). Moreover, the dual modulation of acetylcholine release and synaptic efficacy has been demonstrated

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through the activation of G-protein-coupled muscarinic facilitatory M_1 and inhibitory M_2 receptors on motor nerves (Wessler, 1989; Oliveira et al., 2002). M_1 receptors are also found in skeletal muscles, but their role in neuromuscular transmission is still unclear (Malomouzh et al., 2011a, 2011b). Differences between M_1 and M_2 receptors activation depend on the nerve stimulation pattern and crosstalk with presynaptic adenosine A_{2A} receptors (Oliveira et al., 2002; Oliveira and Correia-de-Sá, 2005). Adenosine can be released from nerve terminals, muscle fibers, and perisynaptic Schwann cells (Cunha and Sebastião, 1993; Santos et al., 2003; discussed in Todd and Robitaille, 2006), in parallel with the formation of nucleoside from the hydrolysis of adenosine triphosphate released synchronously with acetylcholine in a frequency-dependent manner (Smith, 1991; Magalhães-Cardoso et al., 2003). Muscle paralysis induced by the irreversible muscle-type nicotinic receptor antagonist α-bungarotoxin reduces adenosine release from skeletal muscle fibers to levels beyond those required to regulate nerve activity (Noronha-Matos et al., 2011). We hypothesized that cholinoceptor activation that leads to adenosine release from nerve terminals and skeletal muscle might be involved in the paradoxical neostigmine-induced TOF_{fade}. Thus, the involvement of muscarinic and adenosine receptors in neostigmine-induced TOF_{fade} was investigated in the rat phrenic nerve diaphragm.

2. Material and methods

The Ethics Committee for Experimental Studies of the State University of Maringá approved the procedures used in the present study (2600/2011-PRO). Male Wistar rats (250 g) were anesthetized with an intramuscular injection of ketamine (40 mg/kg) and xylazine (8 mg/kg). Phrenic nerve diaphragm muscle preparations were isolated and assembled according to Bülbring (1946). Each preparation was immersed in a 20 mL chamber that contained Krebs buffer (110 mmol/L NaCl, 4.7 mmol/L KCl, 3 mmol/L $CaCl_2$, $MgCl_2$ 1.3 mmol/L, 25 mmol/L $NaHCO_3$, 1 mmol/L KH_2PO_4, and 11.1 mmol/L glucose) at 37 °C, which was continuously gassed with a mixture of oxygen (95%) and carbon dioxide (5%). Hemidiaphragms were connected to an isometric force transducer (Grass FT 03, Grass Instruments Division, West Warwick, RI, USA). Muscle contraction responses were continuously recorded at a resting tension of 50 mN

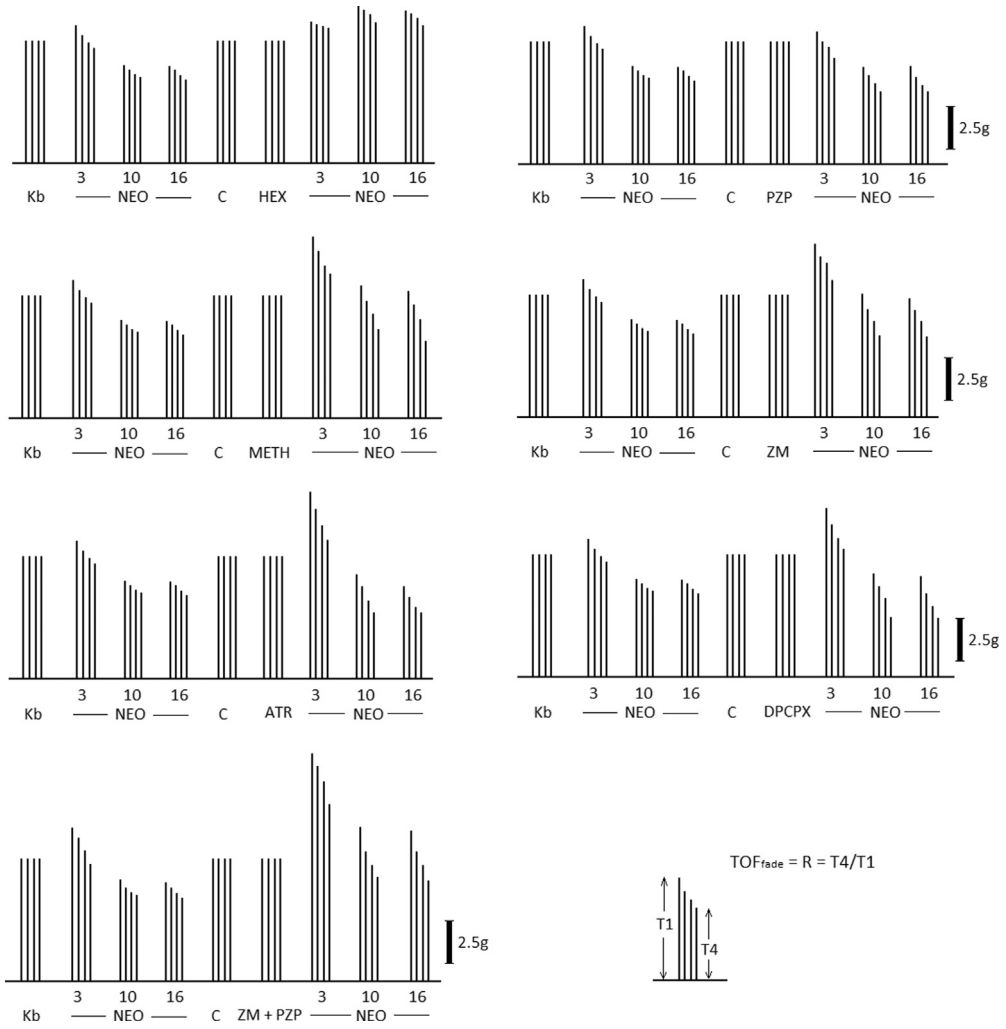

Fig. 1. Typical trace recordings of nerve-evoked hemidiaphragm contractions obtained during TOF stimulation in the absence (Kb, Krebs buffer) and presence of neostigmine (NEO, 1 μM) alone. Records obtained after combined administration of NEO with hexamethonium (HEX; 200 μM), pirenzepine (PZP, 10 nM), methoctramine (METH, 1 μM), 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl)phenol (ZM241385; ZM, 10 nM), atropine (ATR, 0.20 μM), and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX, 2.50 nM) are showed. Records obtained with administration of NEO after the addition of ZM241385 and PZP in the bath are also displayed. The equivalent tension of 2.50 g is indicated on the right. TOF_{fade} was calculated as the ratio (*R*) between the fourth tension recorded at the end (T_4) and the initial tension (T_1) at the beginning of TOF stimulation.