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MODULATION OF DOPAMINE-MEDIATED FACILITATION AT THE NEUROMUSCULAR JUNCTION OF WISTAR RATS: A ROLE FOR ADENOSINE A_1/A_{2A} RECEPTORS AND P2 PURINOCEPTORS

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Abstract—This study aims to understand how dopamine and the neuromodulators, adenosine and adenosine triphosphate (ATP) modulate neuromuscular transmission. Adenosine and ATP are well-recognized for their regulatory effects on dopamine in the central nervous system. However, if similar interactions occur at the neuromuscular junction is unknown. We hypothesize that the activation of adenosine A_1/A_{2A} and/or P2 purinoceptors may influence the action of dopamine on neuromuscular transmission. Using the rat phrenic nerve hemi-diaphragm, we assessed the influence of dopamine, adenosine and ATP on the height of nerve-evoked muscle twitches. We investigated how the selective blockade of adenosine A_1 receptors (2.5 nM DPCPX), adenosine A_{2A} receptors (50 nM CSC) and P2 purinoceptors (100 μM suramin) modified the effects of dopamine. Dopamine alone increased indirect muscle contractions while adenosine and ATP either enhanced or depressed nerve-evoked muscle twitches in a concentration-dependent manner. The facilitatory effects of 256 μM dopamine were significantly reduced to 29.62 ± 2.79% or 53.69 ± 5.45% in the presence of DPCPX or CSC, respectively, relative to 70.03 ± 1.57% with dopamine alone. Alternatively, the action of 256 μM dopamine was potentiated from 70.03 ± 1.57, in the absence of suramin, to 86.83 ± 4.36%, in the presence of suramin. It can be concluded that the activation of adenosine A_1 and A_{2A} receptors and P2 purinoceptors potentially play a central role in the regulation of dopamine effects at the neuromuscular junction. Clinically this study offers new insights for the indirect manipulation of neuromuscular transmission for the treatment of disorders characterized by motor dysfunction.
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Key words: acetylcholine, dopamine, adenosine triphosphate, adenosine, neuromuscular junction, neuromodulators.

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Abbreviations: ATP, adenosine triphosphate; CGRP, calcitonin gene-related peptide; CSC, 8-(3-Chlorostyryl) caffeine; DMSO, dimethyl sulphoxide; DPCPX, 1,3-Dipropyl-8-cyclopentylxanthine; PKA, Protein Kinase A; SNARE, Soluble NSF (N-ethylmaleimide sensitive factor) Attachment Protein Receptor; VIP, vasoactive intestinal peptide.

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INTRODUCTION

Neuromuscular transmission is an elaborate process recruiting the neurotransmitter acetylcholine at the motor end-plate to execute muscle contraction. Efficient transmission is ensured by the precise regulation of acetylcholine release by cholinergic and non-cholinergic receptors such as adenosine, adenosine triphosphate (ATP) (Wood and Slater, 2001) and potentially dopamine receptors (Ganguly and Das, 1979; Pierce et al., 2007; AlQot et al., 2015) located at the neuromuscular junction. The understanding of how these receptors cooperate to match acetylcholine release to neuromuscular demand is not fully developed, and its elucidation can help provide novel insights into the complex process of cholinergic signaling at the neuromuscular junction.

At the neuromuscular junction, endogenous ATP is stored and released with acetylcholine from the motor nerve terminal as well as from activated muscle fibers (Vizi et al., 2000; Santos et al., 2003). ATP can activate P2 purinoceptors located presynaptically on motor nerve terminals and postsynaptically (Henning, 1997). Numerous reports have mounted documenting the effects of ATP at the neuromuscular junction. While some studies suggested an ATP-induced enhancement of acetylcholine release (Salgado et al., 2000), other studies demonstrated a predominant inhibitory effect (Giniatullin and Sokolova, 1998). ATP was believed to mediate these effects either directly via the P2 purinoceptors (Salgado et al., 2000; De Lorenzo et al., 2006) or indirectly through its hydrolytic product, adenosine, and the subsequent activation of adenosine A_1/A_{2A} receptors (Correia-de-Sá et al., 1991; Cunha et al., 1996; Silinsky et al., 1999).

On the other hand, adenosine appears to be derived from three different sources at the motor end-plate; nerve cells, activated muscle fibers and from the catabolic breakdown of ATP (Smith, 1991; Cunha and Sebastião, 1993). Similar to ATP, adenosine plays a dual role in the regulation of neuromuscular transmission, whereby it can either decrease or increase acetylcholine release via presynaptic adenosine-A_1 and adenosine-A_{2A} receptors, respectively (Correia-de-Sá et al., 1996).

In both cases of ATP and adenosine, the depressant effects on acetylcholine release, are predominant under physiologic conditions at the neuromuscular junction (Silinsky et al., 1990; Giniatullin and Sokolova, 1998).

In the case of dopamine, it is neither released nor stored at the neuromuscular junction, however,

presynaptic and postsynaptic dopamine D_1-like receptors have been identified at the motor end-plate (Ganguly and Das, 1979; Pierce et al., 2007). Recently we have shown that dopamine can potentiate neuromuscular transmission by activating presynaptic dopamine D_1-like receptors, rather than β-adrenoceptors, and that this effect may involve enhanced calcium release via the ryanodine calcium release channels (AlQot et al., 2015).

Centrally, dopamine in conjunction with acetylcholine, adenosine and ATP, contribute a fundamental role to the regulation of motor function (Decker and Mcgaugh, 1991; Missale et al., 1998; Franco et al., 2007). We propose that a similar paradigm may exist at the level of the neuromuscular junction, whereby acetylcholine release is modified accordingly via interactions between dopamine, adenosine and ATP receptors located on pre- and/or postsynaptic nerve terminals. We hypothesize that the enhanced neuromuscular transmission induced by dopamine may be modified by adenosine A_1, adenosine A_{2A} receptors, and P2Y purinoceptors.

EXPERIMENTAL PROCEDURES

Materials

The test drug agents; adenosine, adenosine 5′-triphosphate dipotassium salt dihydrate, dopamine hydrochloride, 1,3-Dipropyl-8-cyclopentylxanthine (DPCPX; selective adenosine A_1 receptor antagonist), 8-(3-Chlorostyryl) caffeine (CSC; selective adenosine A_{2A} receptor antagonist) and suramin sodium salt (P2 purinoceptor antagonist) were purchased from Sigma Aldrich. Gallamine tri-ethiodide (non-depolarizing neuromuscular blocker) was obtained from Alexandria Pharmaceutical Company. The solvent dimethyl sulphoxide (DMSO) was purchased from Loba Chemie. Kreb's solution chemicals were procured as follows; sodium chloride and potassium chloride from Iso-Chem. Co, magnesium sulfate heptahydrate from Fischer Scientific Co., calcium chloride anhydrous from Merck, potassium dihydrogen phosphate from El Nasr Pharm. Co and sodium bicarbonate from Chemajet Pharm. Co.

The solvent DMSO was used to solubilize the CSC and DPCPX, both of which are water insoluble. While adenosine is water soluble the higher concentrations used in this study required DMSO as a solvent as per the manufacturer's instructions. Alternatively, dopamine, ATP and suramin were prepared in double distilled water following the supplied product data sheet.

Animals

Male albino Wistar rats with average weight 160–250 g were housed in standard conditions in the Animal Facility at the Faculty of Pharmacy, Alexandria University. Dietary servings constituted of chow and water was allowed *ad libitum*. All experimental protocols were approved by the Animal Care and Use Committee/ACUC (Faculty of Pharmacy, Alexandria University) and strictly abided to the Standard Principles of Laboratory Animal Care.

Rat phrenic hemi-diaphragm preparation

The isolation of the rat phrenic hemi-diaphragm preparation was performed according to the method described by Bulbring (1946). The rats were sacrificed using a blow to the head with subsequent cervical dislocation and exsanguination. A fan-shaped muscle with an intact phrenic nerve was isolated from the left side and transferred to a petri dish containing Krebs' solution (NaCl 95.5, KCl 4.69, $CaCl_2$ 2.6, $MgSO_4 \cdot 7H_2O$ 1.18, KH_2PO_4 2.2, $NaHCO_3$ 24.9, and glucose 10.6 mM) and aerated with carbogen (95% oxygen and 5% carbon dioxide).

Electric Stimulation and measurement of the muscle's force displacement/twitch tension

The isolated left phrenic hemi-diaphragm was transferred to a 75 ml organ bath where the muscle's base (attached rib) was fixed by two pins which served as an electrode for direct muscle stimulation. The apex (tendinous part) of the muscle was attached to a thread and connected to a Grass force displacement transducer (model FT-03C) and the phrenic nerve was positioned in contact with a pair of platinum electrodes for indirect stimulation. Care was taken so as not to pull the nerve or allow it to dry. The transducer was subsequently connected to a computerized data acquisition system with Lab Chart-7 Pro software (Power Lab 4/35, model ML 866/P; AD Instruments Pty Ltd, Castle Hill, Australia) via an MLAC11 Grass adapter. The bathing solution (Kreb's solution) was maintained at a constant volume of 50 ml, constant temperature of 37 °C and continuously aerated with 95% oxygen and 5% carbon dioxide.

Electric stimulation was induced using the Grass electronic stimulator (model S48). We used rectangular pulses, 0.5 ms in width, at a frequency of 0.1 Hz with supramaximal voltage of 3–5 V and 20–50 V for indirect and direct muscle stimulation, respectively.

Prior to each experiment, the preparations were stretched at one gram tension and conditioned to electrical stimulation for 20 min. In studies involving direct muscle stimulation, 224 μM gallamine triethiodide was added to the bathing fluid to block interference from the phrenic nerve. Alternatively, for experiments with indirect muscle stimulation the preparation was treated with 67–134 μM gallamine triethiodide to reduce nerve-evoked muscle twitches to half their original height and increase muscle sensitivity.

Time-matched controls were performed using the vehicle instead of the test agent and each agonist/antagonist concentration was allowed 10 min contact time. Results are expressed as percentage change in amplitude of indirect muscle twitches relative to control.

Experimental protocols

For each experiment six animals were used to obtain sufficient data to minimize variability and demonstrate significance.

The effect of exogenous ATP on skeletal muscle performance. In this set of experiments, the effects of

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