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Exogenous AMP decreases EPP amplitude at the frog neuromuscular junction

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ABSTRACT

The communication between the motor nerve and the skeletal muscle can be disrupted by repetitive stimulation of the nerve, a phenomenon known as presynaptic depression. This depression is caused by the co-release of ATP with the neurotransmitter acetylcholine (ACh) during nerve stimulation, as the ATP and its hydrolysis product inhibit further release of ACh. Using the sartorius muscle and vagus nerve of *Rana pipiens pipiens*, we used intracellular recording techniques to test the effects of exogenous AMP on neurotransmitter release. We found that, like ATP and adenosine, AMP significantly decreases EPP amplitude. Therefore, we believe that AMP is either: a) converting to ATP or adenosine and then acting through these pathways or b) binding to unidentified AMP-specific receptors to cause a decrease in ACh release.

INTRODUCTION

The motor nerve communicates with skeletal muscles through the skeletal neuromuscular junction, but this communication is easily disrupted by even short periods of repetitive nerve stimulation. Repetitive stimulation decreases the amount of the neurotransmitter acetylcholine (ACh) released at the neuromuscular junction (NMJ), leading to presynaptic depression (Redman and Silinsky 1994). While the more advanced phase of presynaptic depression is caused by the exhaustion of the available supply of ACh, there is a phase of presynaptic depression that occurs despite evident availability of ACh (Redman and Silinsky 1994). This early phase of depression appears to be caused by purinergic receptors that modulate the release of ACh. Silinsky and Redman (1996) found that in the cutaneous pectoral nerve-muscle of the frog, single nerve impulses that stimulate the release of ACh at the neuromuscular junction simultaneously stimulate the release of ATP, which in turn mediates prejunctional depression by depressing release of ACh. There has been some debate over which purine is actually responsible for prejunctional depression: while the addition of exogenous ATP has been shown to inhibit ACh release, Silinsky and Redman (1994) found that it was adenosine, the hydrolysis product of ATP, and not actually ATP that inhibited ACh release. However, Giniatullin and Sokolova (1998) recently found that the addition of exogenous ATP depresses the release of ACh at the frog neuromuscular junction even when adenosine receptors are blocked. This suggests that the two purines independently affect ACh release, acting through independent receptors.

While it is known that ATP and its breakdown product adenosine play key roles in modulating neurotransmitter release, little is known about the effects that other breakdown products of ATP – ADP and AMP – might have on this process. In this study, we used electrophysiological recording techniques to observe the effect of exogenous AMP on neurotransmitter release at the frog NMJ. We report that AMP decreased the amplitude of end plate potentials (EPPs) observed in the frog sartorius muscle.

MATERIALS AND METHODS

Frog Preparation and Solutions

All experiments were performed on a sartorius muscle (with vagus nerve attached) dissected from a common grass frog, *Rana pipiens pipiens,* that had been double-pithed. We pinned the muscle in a dish, covering it with fresh Ringer Solution (112 mM NaCl, 3.2 mM KCl, 2.7mM $CaCl_2$-$2H_20$, 0.5 mM Na_2HPO_4, 2.0mM Tris Buffer, and 2.0mM D-glucose, pH=7.2). All drugs applied to the muscle were diluted into fresh Ringer solution. Ringer solution containing curare (7 μM) was applied to prevent the muscle from contracting in response to a stimulus several times threshold, and control readings were taken in this curare/Ringer solution. Thirty μL of AMP, prepared before the experiment and frozen as 100mM aliquots, were added to 30mL of the curare/ Ringer solution and mixed for 10 minutes before experimental readings were taken. For two muscles, after recording AMP readings, the muscle was washed with the curare/Ringer solution for 10 minutes and more readings were taken in this control solution (henceforth referred to as “rinse” readings).

Recording Techniques and Data Analysis

The vagus nerve was suctioned into a suction electrode in order to stimulate it with a G*RASS* SD9 Stimulator, which delivered square depolarizing pulses of 0.2-0.8V for 0.075 s at 4.0Hz. We searched for end-plate potentials (EPPs) using standard intracellular recording techniques with glass recording electrodes filled with 3M KCl (with ~5-20MΩ resistance). The average resting membrane potential of cells we recorded from was between -50 and -90mV. EPPs were recorded using Scope v.3.6.9 and MacLab 6.0, and the amplitude of each reported EPP value represents eight individual readings averaged together. Data were analyzed using a two-sample t-test in Minitab-13 ($p<0.05$ was considered significant).

RESULTS

The presence of exogenous AMP significantly decreased mean EPP amplitude in comparison to control readings ($p<0.001$, $n=4$) (Fig 1). In comparison to rinse readings, the mean EPP amplitude was lower for the AMP treatment; however, the change was not quite significant, probably due to low sample size ($p=0.052$, $n=2$). We found that neither the time since dissection to recording of EPP nor the stimulation voltage at which the EPP was recorded significantly affected the amplitude of the EPP ($p=0.229$, $p=0.501$).

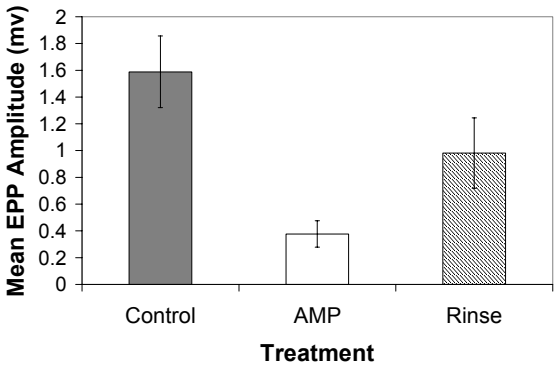

Figure 1. Effect of exogenous AMP on mean EPP amplitude. AMP significantly decreased mean EPP amplitude as compared to control (n=4). After rinsing off the AMP solution, the mean EPP increased but was not significantly different from AMP or control readings (n=2). ($P<0.05$ is considered significant, error bars represent +/- 1 S.E.)

DISCUSSION

Exogenous AMP was found to significantly reduce the amplitude of EPPs at the frog NMJ, mirroring the reported inhibitory effects that ATP and adenosine have on neurotransmitter release (Meriney and Grinnell 1991, Redman and Silinsky 1994). While early research indicated that ATP hydrolyzed to adenosine to produce the observed response, Giniatullin and Sokolova (1998) found that both ATP and adenosine separately affected ACh release acting through separate receptors. This is supported by studies in perisynaptic glial cells, where both ATP and adenosine independently affect neurotransmitter release (Robitaille 1995). Accordingly, AMP probably affects the frog NMJ by one of three methods: 1) it hydrolyzes to adenosine, 2) it is phosphorylated to ATP, or 3) it directly affects ACh release. The second possibility seems to be the least likely, because of the time involved in phosphorylation of AMP to ADP to ATP, and because the muscle would probably run out of energy for the phosphorylation process soon after the dissection. In contrast, the first possibility is more likely because dephosphorylation is an exothermic process that would not be impaired by a limited supply of energy in a dying muscle preparation. Furthermore, Smith (1991) found that the breakdown of adenine nucleotides to adenine is a fairly rapid process. Rapid hydrolization would deplete the amount AMP present, which argues against our third possibility as AMP would be hydrolyzed before it was able to bind to a specific receptor. However, Giniatullin and Sokolova (1998) argue that the rate of hydrolization is slow compared to the lifetime of ACh, so then a scenario where AMP acts through its own receptors would be possible. To determine which scenario is most viable, further study should investigate the effect of exogenous AMP on the neuromuscular junction when adenosine receptors are blocked or when AMP is prevented from degrading to adenosine. Once the pathway through which AMP acts is known, the information could be used to provide treatment for patients with diseases such as myasthenia gravis, which causes extended prejunctional neuromuscular depression (Redman and Silinsky 1994).

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