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Blocking of muscarinic receptors does not affect post tetanic response in frog neuromuscular junction.

BRIAN MARION, KIMBERLY MCLEAN, and ANA NAGEL
Department of Biology, Grinnell College, Grinnell, Iowa

ABSTRACT

Post-tetanic potentiation (PTP) is an often-studied form of synaptic plasticity that is associated with motor learning. Although the exact physiological mechanism is as yet unknown, there is some evidence that muscarinic receptors may play a role in this process, as muscarinic antagonists have been shown to prevent the induction of long-term potentiation by tetanic stimulation. However, after observing the effects of the general muscarinic inhibitor atropine on the end-plate potentials (EPPs) of the frog sartorius muscle, we report preliminary data suggesting that muscarinic receptors do not play a significant role in the induction of PTP. Observations of the amplitude of the EPPs suggest that muscarinic receptors do play a role in the modulation of neurotransmitter (NT) release in the neuromuscular junction (NMJ), as evidenced by the decrease in EPP amplitude in the presence of the muscarinic antagonist atropine.

INTRODUCTION

Post-tetanic potentiation (PTP) is a physiological form of synaptic plasticity which may be involved with motor learning (Calabresi et al., 1996). Although the precise mechanisms behind PTP are as yet unknown, muscarinic receptors may play a role in this important physiological process. This is suggested by data demonstrating that muscarinic receptor antagonists may prevent the induction of long-term potentiation by tetanic stimulation (Calabresi, 2002). These receptors, located in the neuromuscular junction (NMJ), have been shown to play a role in controlling neurotransmitter (NT) release (Forster and Blaha, 2000; Kimura and Baughman, 1997), as well as to mediate muscle contraction (Nelson et al., 1996; Parkman et al., 1999; Wrzos et al., 2004), indicating that they play an important role during the tetanic stimulation that creates PTP.

Muscarinic receptors mediate muscle contraction via regulation of acetylcholine (ACh) release at the NMJ. This is most likely achieved by the modulation of Ca^{+2} channels, which alter the Ca^{+2} concentrations that determine whether ACh release will occur (Shapiro et al., 1999; Shapiro et al., 2001). These receptors are able to produce a variety of effects on muscle contraction, due to the specializations of muscarinic subtypes M1-M5. Of these subtypes, M3 receptors have been found to play a role in the inhibition of ACh release, and M1 receptors have been found to play a role in the enhancement of ACh release at the lizard NMJ (Graves et al., 2004; Slutsky et al., 2001). Another receptor, the M2 subtype, has been found to play a role in the process that terminates ACh release in the frog NMJ (Slutsky et al., 1999; Slutsky et al., 2001; Slutsky et al., 2003). Any or all of these receptors may affect the mechanisms responsible for creating PTP, as evidenced by the fact that atropine (50 μM), a non-selective muscarinic antagonist which binds to all muscarinic subtypes, can cause changes in the sequence dependence of PTP (Seki et al., 2001). In order to specifically observe the effects of atropine on PTP, we have investigated its effects on end-plate potentials (EPP) in the sartorius muscle preparation of *Rana pipiens*. After observing the EPP with exposure to atropine, we report preliminary results that muscarinic receptors do play a role in modulating the magnitude of EPPs, and that atropine was not found to cause any significant changes in PTP at the frog NMJ.

MATERIALS AND METHODS

Set up of muscle preparation

We began by dissecting out the sartorius muscle with about one to two mm excess of its main enervating bundle out of our *Rana pipiens* specimen. The muscle was pinned down in a dish of frog Ringer's solution with glucose. A suction electrode was then used to determine the threshold at the nerve bundle by stimulating at progressively larger voltages until motor activity was observed. Thresholds ranged between 0.4 volts to 0.8 volts. The muscle was then bathed in either a 10μM solution of curare in Ringer's solution for control, or a 10μM atropine with 10μM curare in Ringer's solution for our experimental condition. After waiting for 15 to 20

minutes for the toxins to take effect, end plates were found using intracellular recording with microelectrodes.

Collecting Data

We used the program Scope v. 3.6.3. to locate and record traces of the endplate potentials. Once an endplate was located, we recorded as the nerve was stimulated every 5 seconds at twice the threshold voltage. After taking such readings for one minute, we stopped recording and gave a high frequency stimulus (200 stimuli/sec) for 5 seconds. Recordings were then resumed at one stimulus every five seconds until the traces seemed to return to their pre-tetanic-stimulation state (about 5 minutes).

Analyzing Data

The initial amplitude and timing data were collected in Scope v. 3.6.3. The amplitude data was then transferred into Microsoft Excel for the calculation of descriptive statistics. Finally, the amplitude data was transferred into Minitab to standardize the scale of the data and to test the differences between the data sets for significance.

RESULTS

We found that while the inhibition of all muscarinic receptors does cause an overall decrease in the amplitude of the EPPs, it does not appear to significantly affect PTP. Initial statistical analysis showed significant differences between the two control data sets ($p \leq 0.001$); we therefore decided to standardize all of our data to the same scale (mean=0, SD=1). After scaling, we found no significant differences between the two controls ($p=1.00$), and no significant difference between the sets of experimental data ($p=1.00$). However, there was also no significant difference found when the controls were compared to the experimental data ($p=1.00$).

When comparing our control data sets to those from the experimental condition with atropine, we did find some significant differences. There was a reduction of the EPP amplitude in the presence of atropine; but aside from this, we found no other specific differences in the pattern of PTP between the two conditions. To characterize our observation we looked specifically at the maximum and minimum amplitudes of each data set. The minimum amplitude approximately represents the normal EPP before the high frequency stimulus. The maximum amplitude represents the highest response that we got during the PTP response. The controls had maximum amplitudes of 4.29 volts and 12.13 volts and minimum of 1.07 volts and 2.99 volts. The experimental preps had maximum amplitudes of 3.43 volts and 1.56 volts. The minimum amplitudes were 0.67 volts and 0.28 volts. After employing the same scaling procedure used to compare EPPs, none of the PTPs from any data set showed any significant differences from the others; however, there were a few non-significant trends between the experimental and control conditions.

	Maximum	Minimum
Control 1	4.29	1.07
Control 2	12.13	2.99
Experimental 1	3.43	0.67
Experimental 2	1.56	0.28

Table 1. A comparison of the maximum and minimum amplitudes of the control and experimental data. The experimental data was observed to be decreased overall rather than just in PTP.

Figure one better shows the differences between the amplitude data for the different data sets. The controls tend to show a higher amplitude change after tetanic stimulation, and have a much more distinctive response. In both the controls there is a noticeable dip after the high frequency stimulus (see arrow, figure 1), followed by a steep but steady increase, and finally a slow decrease. The experimental data tends to have smaller amplitudes overall than the control. The experimental data still shows a slight dip, but it is harder to see, and the post-tetanic response appears flatter and more gradual.

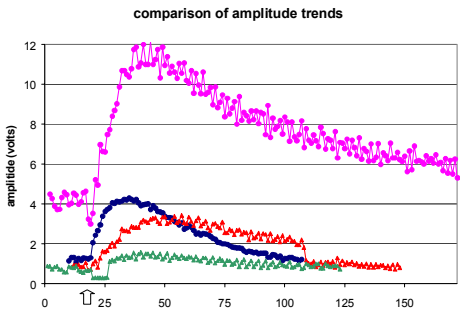

Figure 1. The pink and dark blue lines (with circle data points) depict the control amplitudes data. The red and dark green lines (with the triangular data points) show the experimental, with atropine, amplitudes data. The amplitudes are plotted against an arbitrary x-axis so that they would all be approximately on the same scale. The arrow on the x-axis shows the point in which the high frequency stimulus was given for each run. Both the control runs are higher and their post tetanic potentiation responses have a more distinct curve.

DISCUSSION

In general, we observed that atropine causes a decrease in the amplitude of EPPs (Table 1), and that PTP is not significantly changed in the presence of atropine (Figure 1). This is in accord with previous studies suggesting that muscarinic receptors play a critical role in the regulation of NT release (Forster and Blaha, 2000; Kimura and

Baughman, 1997), because EPPs are in part an indirect measurement of the release of NTs. Our data also supports studies suggesting that muscarinic receptors play a role in the mediation of muscle contraction (Nelson et al., 1996; Parkman et al., 1999; Wrzos et al., 2004). It is likely that the muscarinic receptors facilitate the activity of Ca^{+2} channels, allowing for an influx of Ca^{+2} into the pre-synaptic cell, as suggested by Shapiro et al. (1999; 2001). The influx of Ca+2 then causes the release of ACh through exocytosis, which in turn triggers the depolarization in the post-synaptic cell.

The observed decrease in EPP amplitude (Table 1) is likely due to the blocking of the M1 subtype of muscarinic receptors, rather than the other muscarinic subtypes. M1 subtypes in particular have been observed to facilitate the release of ACh in the NMJ, whereas the M2 and M3 subtypes tend to cause an inhibition of ACh release (Graves et al., 2004; Slutsky et al., 2001). This data therefore suggests that M1 is the predominant muscarinic receptor subtype in the frog NMJ.

This data also suggests that muscarinic receptors do not play a definitive role in synaptic plasticity, which means that they do not facilitate motor learning through PTP. Additional studies should be conducted to determine the prevalence of the M1 subtype in the NMJ and also whether selective M1 antagonists produce decreases in EPP amplitude.

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